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CONTENTS

Dates of Issue of Volume 69

Number 1, for January	January 2, 1942
Number 2, for February	February 2, 1942
Number 3, for March	March 2, 1942
Number 4, for April	April 1, 1942
Number 5, for May	May 1, 1942
Number 6, for June	June 2, 1942
Number 7, for October	October 2, 1942
Number 8, for November	November 2, 1942
Number 9, for December	December 3, 1942

ERRATA

p. 31. For *Sedum Burhamii* read *Sedum Burnhamii.*

p. 184. For *Dendroctonus fontalis* read *Dendroctonus frontalis.*

p. 185. For *Dendroctonus breviconus* read *Dendroctonus brevicomis.*

p. 338. For *Rheinwardtia* read *Reinwardtia.*

p. 376. For *Ranunculus cymbalaria* var. *alpina* read *Ranunculus cymbalaria* var. *alpinus.*

p. 520. For *T. trichophyton* read *T. discoides.*

BULLETIN OF THE TORREY BOTANICAL CLUB
69(1): 1-10 JANUARY, 1942

SECONDARY VASCULAR TISSUES OF THE OAKS INDIGENOUS TO THE UNITED STATES—II. TYPES OF TYLOSES AND THEIR DISTRIBUTION IN ERYTHROBALANUS AND LEUCOBALANUS

SIMON WILLIAMS[1]

The comparative abundance of tyloses in the earlywood pores in the heartwood of white oaks and their absence or sparsity in like structures in the heartwood of red oaks have long been utilized as aids in the separation of these two groups of woods.[2] *Leucobalanus*[3] species are said to possess extremely abundant tyloses, while in species belonging to *Erythrobalanus* they are considered to be sparse or wanting. The diagnostic significance of this discrepancy is admitted to be of limited application. Beyond question tyloses are the rule in most white oaks; their distribution in red oaks is so variable that their presence or absence, when considered alone, if of negligible value in diagnosis. However, in the course of a comparative study of the xylary structure of these two oak subgenera, several morphological types of tyloses were noted; if these are considered in conjunction with the abundance or scarcity of tyloses in oak, several apparently reliable indications of group relationships, in terms of species, are evident.

At the start it is admitted that the varying character of tyloses in *Quercus* and the distribution of the morphological types that can be recognized in the secondary xylem do not permit the segregation of species with the accuracy occasioned by the departures in the nature (shape and wall thickness) of the latewood pores. Nevertheless, this feature is of considerable value, serving as it does as a secondary character in the recognition of *Erythrobalanus* and *Leucobalanus* species and permitting meanwhile of further segregation of species in these two subgenera. It is the purpose of this investigation to illustrate and categorically group the various morphological types of tyloses encountered in a study of oak woods, to point out the structural departures which characterize each group, and to assay the diagnostic value of these tylosic types. Further, toward the end of the paper considerable space is allotted to a discussion of the chemical nature of the tylosic walls in *Quercus*.

[1] The information incorporated in this paper has resulted from research by the author while a member of the Department of Wood Technology, New York State College of Forestry, Syracuse, N. Y. The author is now Research Technologist in the Bureau of Industrial Chemistry, University of Texas, Austin, Texas.

[2] Tyloses are uncommon in the latewood pores in *Quercus;* in this position they appear to be more frequent in *Erythrobalanus* than in *Leucobalanus.*

[3] The limits of *Erythrobalanus* and *Leucobalanus,* as understood in this discussion, were defined in the first paper of this series (2).

[THE BULLETIN for December (68: 609–694) was issued December 1, 1941]

TYPES OF TYLOSES IN THE XYLEM OF QUERCUS

Recognition of types of tyloses in *Quercus* is dependent in large measure on a variation in the number present in the cavities of the earlywood vessels and hence on the size of the tyloses, and on the thickness of the tylosic wall; stratification of the wall and pits, when present, serve as secondary characters. Three classes of tyloses may be recognized on the basis of the features enumerated above, as is indicated in table 1 and illustrated in figures 1 to 10, inclusive.

TABLE 1

Types of tyloses occurring in Quercus species

A—Tylosic walls extremely thin (under 2 microns)
   1—Tyloses relatively sparse per vessel cavity (figs. 1 and 8).
   2—Tyloses relatively abundant per vessel cavity (figs. 2 and 9).

B—Tylosic walls of "normal thickness" (3 to 5 microns); tyloses relatively sparse to moderately abundant per vessel cavity (figs. 3, 6, and 7).

C—Tylosic walls thick to extremely thick (5 to 50 microns), not infrequently stratified and pitted; tyloses sparse to abundant per vessel cavity (figs. 4, 5, and 10).

DISTRIBUTION OF TYLOSIC TYPES IN LEUCOBALANUS

Information on the types of tyloses and the abundance of tyloses in *Leucobalanus* are incorporated in table 2.

In *Leucobalanus*, the general statement can be made that tyloses are extremely abundant; they are usually present in all of the earlywood pores with the possible exception of a few of the last-formed pores, and are predominantly of Type B (see table 1). Exceptions occur to this rule, but they are few in number. *Q. bicolor* Willd., a chestnut oak, appears to possess tyloses of Type A, but Type B tyloses (of the kind which characterize *Q. alba* L.) may be present. Tyloses are sporadic in *Q. montana* Willd., some samples being almost devoid of these structures. *Q. sadleriana* R. Br. Campst., the only chestnut oak on the West Coast, had very few tyloses in the one sample studied. The remaining chestnut oaks, *Q. prinus* L. and *Q. muhlenbergii* Engel., possess abundant Type B tyloses, however, so it is difficult to generalize about the type common to this group. Nevertheless, there does seem to be a nebulous relationship in the nature of the tyloses in the chestnut oaks which further study might substantiate.

DISTRIBUTION OF TYLOSIC TYPES IN ERYTHROBALANUS

In table 3 are embodied data on the types of tyloses and the abundance of these structures in *Erythrobalanus.*

Explanation of figures 1–5

FIG. 1. *Q. bicolor* Willd. Illustrating Type A1, in cross section. FIG. 2. *Q. velutina* Lam. Illustrating Type A2, in cross section. FIG. 3. *Q. stellata* Wang. Illustrating Type B, in cross section. FIG. 4. *Q. arizonica* Sarg. Illustrating Type C, in cross section. FIG. 5. *Q. emoryi* Torr. Illustrating Type C, in cross section. Note pits in the tyloses walls. All × 225.

Figure 1

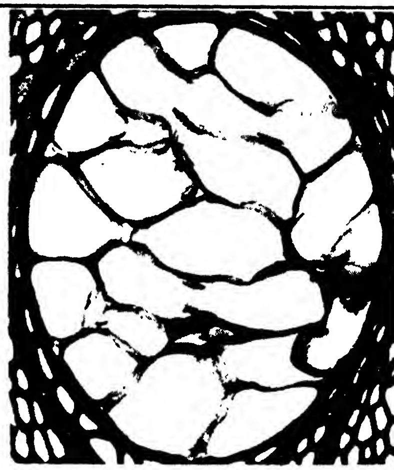

Figure 2

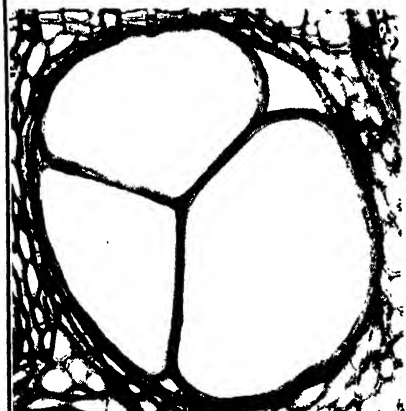

Figure 3

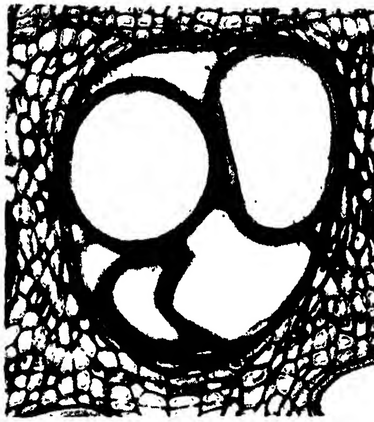

Figure 4

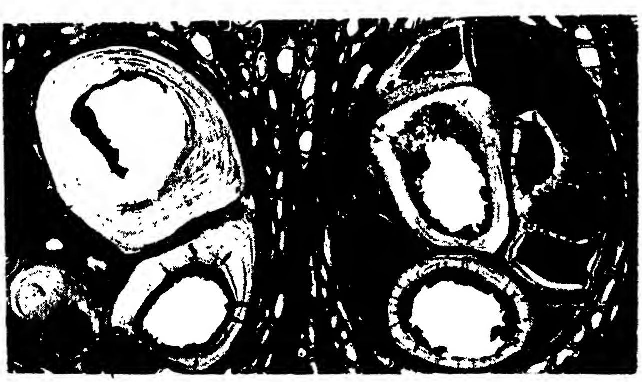

Figure 5

TABLE 2

Types and abundance of tyloses in Leucobalanus

Species	Type	Abundance
Q. alba	B	the rule
Q. bicolor	A1	the rule
Q. durandii	B	the rule
Q. garryana	B	the rule
Q. breweri	B	the rule
Q. lobata	B (A2)[a]	the rule (A2 very rare)
Q. lyrata	B	the rule
Q. macrocarpa	B	the rule
Q. montana	A1 and B	sporadic
Q. muhlenbergii	B	the rule
Q. prinus	B	the rule
Q. sadleriana	B	extremely sparse
Q. stellata	B	the rule
Q. utahensis (gambellii)[b]	B	the rule

[a] The type indicated in parentheses occurs only sporadically and never in any abundance.

[b] The nomenclature of this and closely related species is extremely confusing. Two of the samples were submitted as *Q. gambellii* Nutt. Two others were identified as *Q utahensis* Rydb. Investigation revealed that there is no certain way of distinguishing the various species and varieties, although *Q. gambellii* is generally regarded as the shrubby form. To simplify this taxonomic problem for use in this study, all samples submitted were considered under one heading.

Analysis of this information brings out the following facts:

1. Nine red oaks have tyloses throughout the heartwood at all times; the the following statements may be made for these *Quercus* species:
   (a) *Q. cinerea* Michx. and *Q. marilandica* Muench. have tyloses of Type B.
   (b) *Q. kelloggii* Newb. and *Q. ellipsoidalis* Hill are characterized by Type A tyloses, as a rule.
   (c) *Q. arizonica* Sarg., *Q. douglasii* Hook. et Arn., *Q. dumosa* Nutt., *Q. emoryi* Torr., and *Q. engelmannii* Greene possess Type B and Type C tyloses in admixture. Type C is dominant in these oaks with the exception of *Q. douglasii.* Note should be made at this time that these five oaks are xerophytic species occurring in southwestern United States or in California.
2. *Q. oblongifolia* Torr., *Q. hypoleuca* Engel., *Q. reticulata* H. B. K., also xerophytic species from the Southwest, possess Types B and C tyloses in abundance in the heartwood; there is every reason to believe that these Types would be found the rule in these three species were a sufficient number of samples available for examination. Together with the five species enumerated under 1 (c), they therefore may be considered as constituting a natural group of eight species in which tyloses are entirely comparable.
3. In *Q. agrifolia* Nee, *Q. chrysolepis* Liebm., *Q. wislizenii* A. DC., *Q. myrti-*

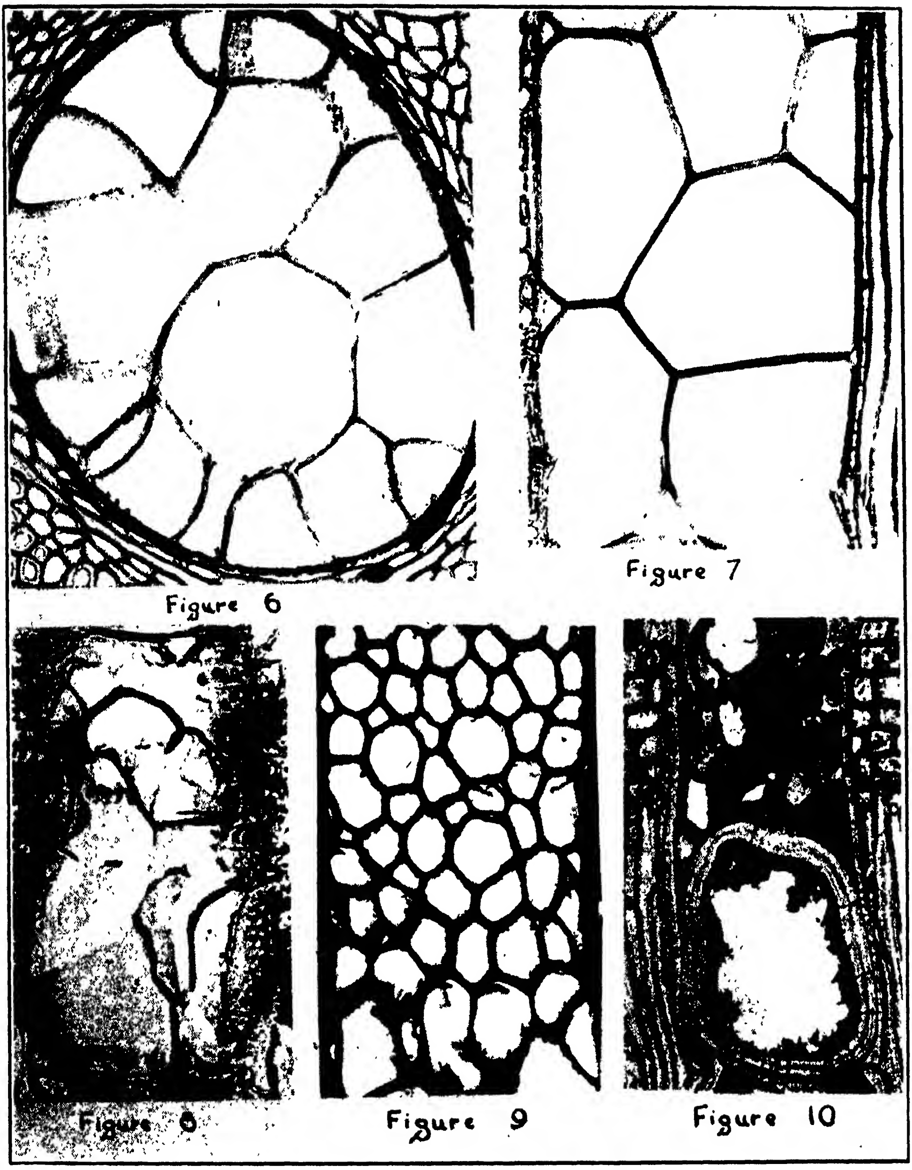

FIG. 6. *Q. prinus* L. Illustrating Type B, in cross section. Note that tyloses are more abundant than in figure 3. FIG. 7. *Q. stellata* Wang. Illustrating Type B, in longitudinal section. FIG. 8. *Q. bicolor* Willd. Illustrating Type A1, in longitudinal section. FIG. 9. *Q. velutina* Lam. Illustrating Type A2, in longitudinal section. FIG. 10. *Q. emoryi* Torr. Illustrating Type C, in longitudinal section. All × 225.

TABLE 3

Types and abundance of tyloses in Erythrobalanus

Species	Type	Abundance
Live Oaks		
Q. agrifolia	A2	sparse
Q. arizonica	C (B)[a]	the rule
Q. chrysolepis	absent	....
Q. douglasii	B and C	the rule
Q. dumosa	B and C	the rule
Q. durata	B and C	sparse
Q. emoryi	C (A and B)	the rule
Q. engelmannii	B and C	the rule
Q. hypoleuca	B and C	sparse
Q. myrtifolia	absent	....
Q. oblongifolia	B and C	abundant
Q. reticulata	B (C)	sparse
Q. virginiana	A2	sparse
Q. virginiana geminata	absent	....
Q. wislizenii	absent	....
Deciduous Oaks		
Q. borealis maxima	A2 (B)	sparse
Q. catesbaei	A2 (B)	sparse to sporadically abundant
Q. cinerea	B (A2)	the rule
Q. coccinea	A2 (B)	sparse to sporadically abundant
Q. ellipsoidalis	A1	the rule
Q. ilicifolia	absent	....
Q. imbricaria	A2 (A1, B)	sparse
Q. kelloggii	A1 (A2)	the rule
Q. laurifolia	A2	sparse
Q. marilandica	B	the rule
Q. morehus	absent	....
Q. myrtifolia	absent	....
Q. nigra	A2 (B)	sparse
Q. palustris	A2 (B)	sparse
Q. phellos	A2 (B)	sparse
Q. rubra	A2 (B)	sparse to sporadically abundant
Q. rubra leucophylla	A2 (B)	sparse to sporadically abundant
Q. rubra triloba	A2 (B)	sparse to sporadically abundant
Q. shumardii	A2 (B)	sparse to sporadically abundant
Q. velutina	A2 (B)	very sparse
Q. vaccinifolia	B	

[a] The type indicated in parentheses occurs only sporadically and never in any abundance.

folia Willd., and *Q. virginiana* Mill., although tyloses are present in the heartwood, they are extremely sparse and of Type A2.

4. In the eastern deciduous red oaks (*Q. marilandica, Q. cinerea,* and *Q. ellipsoidalis* excepted—see under 1 (a) and (b)—tyloses are very sporadic; they may be abundant in a given ring and sparse or lacking in the next, or absent altogether throughout the sample. When tyloses are present, Type A2 is always predominant in this group. Types B and C may occur but wherever these structures are found in a given ring, Type A2 is always more abundant, even though it is not present in all of the pores.

CHEMICAL COMPOSITION OF TYLOSES

The morphological variation and the fluctuation in the abundance of tyloses that permit of the recognition of tylosic types in American oaks has naturally led to the question as to whether or not these departures find expression in the chemical composition of the tylosic walls. Obviously, owing to the inconstancy of tylosic wall thickness from species to species, as discussed above, quantitative differences must occur. As this is clearly evidenced by microscopic examination, little would be added to the diagnostic significance of the tylosic groups listed in Table 1 by accumulating the exact quantitative data necessary to define these variations in tylosic wall thickness. Therefore, the chemical composition of tyloses as they occur in *Quercus,* discussed below, is based entirely upon qualitative analyses.

Isenberg (1), in a microchemical study of the tyloses occurring in hardwoods, concluded that three kinds appear to exist in terms of chemical composition, viz:

(1) Those with very thin walls composed chiefly of lignin to which are possibly added, on the inside, small irregular patches of cellulose. Example, *Fraxinus* spp.

(2) Those with a thin cellulosic layer within a layer of lignin. Example, *Catalpa speciosa* Ward.

(3) Those which possess a somewhat thicker layer of cellulose within the layer of lignin, with an inner layer of lignin (not visible in untreated sections) in turn deposited on the cellulose. Example, *Q. alba* L.

Isenberg designated this last condition as the oak type. Unfortunately, he examined the tyloses in but three species of oak (*Q. alba* L., *Q. dilata* Lind., and *Q. engelmannii* Greene[4]); because of the information already recorded in this paper relative to the varying nature and abundance of tyloses in domestic oaks, it may rightly be assumed that Isenberg's work was based on too few species of *Quercus* to give any true picture of the qualitative chemical nature of tyloses in this genus. It has therefore seemed advisable to repeat with certain minor changes the qualitative tests run by Isenberg on the species in question and to extend these to a sufficient number of additional species to represent all of the tylosic classes listed in table 1.

Materials and Techniques Employed in Determining the Chemical Composition of Tyloses in Quercus. Tyloses were examined in the following species:

(a) *Q. bicolor* Willd., *Q. montana* Willd., *Q. kelloggii* Newb., and *Q. ellipsoidalis* Hill, representing Type A1. *Q. coccinea* Muench. and *Q. velutina* Lam., representing Type A2.

(b) *Q. stellata* Wang., *Q. alba* L., *Q. cinerea* Michx., and *Q. marilandica* Muench., representing Type B.

[4] Isenberg makes no mention of the fact that *Q. engelmannii* has thick-walled tyloses in admixture with those of the type which feature *Q. alba.*

(c) *Q. emoryi* Torr., and *Q. arizonica* Sarg., representing Type C.

Blocks were cut from the dry heartwood and were extracted with hot water until no further evidence of tannin could be detected by treatment with an aqueous solution of ferric ammonium sulphate. They were then stored in 20 per cent ethyl alcohol until needed. Sections twenty microns thick were prepared from these blocks. These were then examined under polarized light before chemical treatment, and after chemical treatment (a) to remove hemicelluloses and (b) after (a) followed by treatments to remove cellulose or lignin. The examination prior to exposure to chemicals served to indicate the predominantly crystalline and non-crystalline portions of the tylosic wall; the solvent action of the chemicals employed was revealed by the subsequent examination of the material.

The chemical treatment of the sections, to remove hemicellulose, cellulose, and lignin, respectively, were as follows:

1. Removal of hemicellulose. The sections were alternately treated with 0.5 per cent NaOH for one hour and 3 per cent H_2SO_4 for three hours, over a water bath.

2. Removal of cellulose. (a) Treatment as in 1, followed by immersion in an excess of 72 per cent H_2SO_4 on a glass object slide, the reaction observed meanwhile under a microscope. (b) Treatment as in 1, followed by immersion in Schweizer's reagent,[5] the reaction observed meanwhile under a microscope.

3. Removal of lignin. Treatment as in 1, followed by a two minute chlorination (with saturated chlorine water) and then by a two minute immersion in a solution of monoethanolamine in 95 per cent ethyl alcohol (3). The first section examined received one complete treatment, the second two, using the chemicals in the order named, the third three, etc. After treatment the section was thoroughly washed in distilled water and was then examined with a microscope. Finally, it was exposed to 72 per cent H_2SO_4, to gauge the extent to which the lignin had been removed by the above-mentioned reagents. Complete solution of the section in concentrated sulphuric acid, a cellulose "solvent," indicated the fact that the lignin had very nearly been completely removed.

Discussion of the Results of Chemical Treatment of Tyloses in Oak Wood. The test for hemicellulosic material in tylosic walls was entirely negative. Some changes may have resulted but no visible evidence of reaction was discernible other than a slight swelling of the wall and a general increase in the plasticity of the sections. Nor did this treatment have any observable effect on the rate with which cellulose and lignin solvents reacted subsequently.

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[5] It is recognized that cellulose may not be appreciably removed from lignified walls by this reagent.

Treatment with cellulose and lignin solvents yielded more conclusive evidence of the nature of the tylosic wall; this evidence may be summarized as follows:

1. The tyloses of Type A1 proved to be analogous to those of Isenberg's Class I. The bulk of the wall in this instance appeared to consist of lignin which was bounded exteriorly (toward the cavity) by an extremely thin layer of cellulose. Under the polarizing microscope, there was a slight indication that a third layer of lignin was present but this inference could not be proved by removing the cellulose owing to the violence of the sulphuric acid reaction. The extremely thin tylosic wall ruptured long before the xylary cells became dissociated. The cellulosic layer of the wall, in contrast, was sufficiently strong to permit the tylosis to remain intact after complete delignification and appeared to exist as a continuous strip of cellulose rather than of small irregular patches of this substance.

2. The tyloses of Type A2 were similar in all respects to those of Type A1 except that an inner layer of lignin was observable in the tylosic wall while the sections were undergoing treatment.

3. The tyloses of Type B were identical to those of Isenberg's Class 3. The layers of lignin in the tylosic wall bounding the cellulosic layer within and without were clearly discernible in sections swollen with cellulose solvents. The cellulose layer occupied the bulk of the cross-sectional area of the wall.

4. The sclerosed tyloses of Type C with stratified walls were dissimilar to any of the classes listed by Isenberg. All of the wall layers, with the exception of the middle lamella, appeared to be crystalline, that is, cellulosic, throughout. An external (third) layer of lignin was not evident at any time. The thick secondary layer of the wall dissolved rapidly both in 72 per cent H_2SO_4 and Schweizer's Reagent, indicating, particularly in the latter instance, its cellulosic nature.

SUMMARY AND CONCLUSIONS

1. Tyloses are more abundant and a more constant feature in white oaks than in red oaks, but the two subgenera cannot be distinguished with certainty on the basis of the presence or absence of these structures.

2. In general, tyloses in *Leucobalanus* are different structurally from those in *Erythrobalanus.* In white oaks, the tyloses possess walls averaging 3–5 microns in thickness, as a rule, and are moderately abundant; in comparison, two major morphological types of tyloses can be recognized in red oaks, namely, (a) those with walls under 2 microns in thickness, that are abundant or even crowded (Type A2 of table 1) and (b) sclerosed tyloses with walls averaging 5–50 microns in thickness that are frequently stratified and pitted as well, such tyloses ranging from sparse to abundant. (Type C. table 1.)

3. Exceptions to the situation stated under 2 occur but they are not frequent. An intermediate type of tylosis occurs in two species of each subgenus (*Q. montana* Willd., and *Q. bicolor* Willd. in *Leucobalanus; Q. ellipsoidalis* Hill and *Q. kelloggii* Newb. in *Erythrobalanus*) in which the wall is under 2 microns in thickness and the tylosic number is low (Type A1, table 1). Further, two red oaks (*Q. marilandica* Muench. and *Q. cinerea* Michx.) exhibit tyloses of the white oak type throughout the heartwood. The predominant type of tylosis present in all other *Quercus* species conforms to 2.

4. The structural variation of tyloses reaches a peak in *Erythrobalanus;* on the basis of tylosic type and abundance, several subdivisions, each embracing a number of species, can be recognized (see tables 1 and 3).

5. The white oaks, in contrast to the red, do not lend themselves to ready subdivision into groups of species on the basis of the varying morphological nature or abundance of tyloses. Several chestnut oaks appear to be an exception to this rule but since the tylosic features of this group of species exhibit no uniformity, definite deductions cannot be made.

6. The chemical composition of tylosic walls in *Quercus* is more complex than is indicated by the work of Isenberg (op. cit., p. 9). Two distinct types can be recognized, viz:

(a) tyloses with from thin to moderately thick walls (under 5 microns), the latter composed of a central cellulosic layer with thin layers of lignin on its flanks.

(b) tyloses with thick (over 5 microns) and stratified walls which frequently possess pits, the wall composed of a lignified inner layer (middle lamella) followed centripetally by a cellulosic layer, with no layer of lignin to the inside.

7. The physical nature of the tylosic content of *Quercus* woods, occasioned in turn by variations in the wall thickness of tyloses and the number of these per unit of volume, that is, by their size, are more readily decipherable and can be used with much greater ease than the chemical distinctions enumerated in the latter part of this paper.

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THIAMIN CONTENT OF AGAR[1]

DOROTHY DAY

INTRODUCTION

The need for a careful study of agar became obvious during the early stages of another problem. At that time excised roots of tomato, which require thiamin, appeared to grow fairly well in an agar-mineral-sugar medium regardless of the presence of added thiamin. The present report is concerned with the determination by means of the growth of *Phycomyces Blakesleeanus* of the approximate quantity of thiamin or its intermediates in various samples of commercial and purified agar as well as in some samples of filter paper and cheesecloth, materials frequently used in the preparation of culture media.

Agar-agar, so commonly used for solidifying liquid media for the cultivation of micro-organisms, is prepared by drying a hot-water extract of certain marine algae. Because of its origin and the method of preparation it might be expected to contain some of the various growth substances originally present in the algae and perhaps some produced by micro-organisms which might develop in the extract during its desiccation. We should expect also that the amount of the growth substances in various samples of commercial agar would differ, depending upon how much they had been purified by washing or other treatments, and upon the character of any additions which might be made in preparing them for the market.

The presence of thiamin, of biotin, and of unidentified growth substances in commercial agar has been previously reported. Allison and Hoover (1934), using *Bacillus radicicola* as the test organism, detected the presence of Coenzyme R in agar. Coenzyme R was later demonstrated by György and his coworkers (1940) to be identical with biotin or vitamin H. Hawker (1936) found that a scanty growth of *Melanospora destruens* developed on a mineral-glucose medium containing 1.5 per cent agar. No growth occurred in the medium without the agar. These observations were interpreted as indicating the presence of traces of the second of two factors which must be present for the development of this organism. The second factor was later demonstrated to be biotin (1939).

Fries (1938) reported that *Polyporus abietinus* and *Polyporus adustus* grew and formed aerial mycelium on a mineral-sugar medium containing

[1] Contributions from the Department of Botany, Smith College, New Series, No. 8.

I am indebted for many courtesies to the staff of the New York Botanical Garden, where these experiments were performed.

ammonium nitrate and 1.5 per cent agar. Since both of these organisms are thiamin-deficient and do not grow in a liquid medium lacking thiamin, or its pyrimidine intermediate, growth on the agar medium may be taken as prima facie evidence for the presence of thiamin in the agar. Fries observed further that the growth of these two organisms was much less on a medium containing washed agar than on one containing unwashed agar. The washed agar was prepared by extracting discs of 3.0 per cent agar with two changes of distilled water during a 24-hour period.

Fromageot and Tchang (1938) observed that *Rhodotorula Sannei* grew on a mineral-sugar medium containing 2.0 per cent washed agar but failed to grow in the same medium without the agar. This organism does not grow without thiamin or its intermediates in the medium. Their observations indicate that their washed agar contained thiamin or pyrimidine.

Robbins (1939) reported the presence in agar of thiamin or its intermediates as determined by the growth of *Phycomyces Blakesleeanus*. Evidence for the presence of unidentified growth substances (Factor Z) in agar was presented by Robbins (1941a). Robbins (1941b) and Robbins and Ma (1941) found as much as 0.1 mμ mole of biotin per gram in some samples of agar; the biotin was determined by the growth of *Ashbya Gossypii*.

Schopfer and Rytz (1938) found appreciable quantities of thiamin or its intermediates in crude cotton as judged by the growth of *Phycomyces* but little or none in white, bleached cotton.

The literature cited indicates that various growth substances may be present in agar in sufficient amounts to be significant in determining the growth of micro-organisms. However, agar may influence growth for other reasons than its growth-substance content. Its ash, especially the trace elements, may be important for some organisms and under some conditions (Leonian and Lilly, 1940). Rippel (1936) found the colloidal character of the agar significant in favorably affecting the growth of *Azotobacter* and *Aspergillus niger*. Other reasons have been suggested why agar should affect growth, but no attempt will be made here to review such possibilities in detail.

METHODS AND MATERIALS

Phycomyces Blakesleeanus (+) strain was grown at 25° C. on 25 ml. of medium in 125 ml. Erlenmeyer flasks of Pyrex glass cleaned with chromic acid cleaning solution and thoroughly rinsed with tap water and distilled water. The media were sterilized at 15 lb. pressure for 20 minutes. For inoculum several sporangia were broken in a flask containing 25 ml. of sterile distilled water; one drop of this suspension was added to each flask of the medium to be tested. All cultures were grown in quadruplicate.

The basal medium contained per liter of distilled water: 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4 \cdot 7\ H_2O$, 50.0 g. dextrose, 2.0 g. asparagine, and mineral supple-

ments in p.p.m. as follows: B 0.005, Cu 0.02, Fe 0.10, Ga 0.01, Mn 0.01, Mo 0.01, Zn 0.09. The commercial asparagine was purified by treatment with Norit A and recrystallization from alcohol. The thiamin was Merck's synthetic betabion; the pyrimidine was 2-methyl-5-bromomethyl-6-aminopyrimidine dihydrobromide. The amount of thiamin or its intermediates is expressed in millimicromoles (mμ moles).

The agars were Difco granulated bacto-agar, Eimer and Amend agar-agar flakes, and crude, shred agar. For parts of some experiments the granulated and flake agars were thoroughly leached with 5.0 per cent aqueous pyridine, with distilled water, and with alcohol, boiled with alcohol, and dried; these are referred to as "purified agars." The leachings and washings obtained from a particular agar were freed of pyridine and alcohol and concentrated by evaporation on a hot plate; these are referred to as "agar extracts." A portion of the shred agar was electrodialyzed.

Filter paper of two types was also used; one was a low grade paper, No. 613, from Eaton and Dikeman, while the other was "Genuine" Whatman "ashless." These were cut in pieces about one inch square and boiled in distilled water for 20 minutes. The liquid was drained from the paper and concentrated by evaporation on a hot plate. Two samples of cloth, commonly known as "cheesecloth" and as "tobacco cloth" respectively, were treated similarly. These liquids are referred to as paper and cloth extracts.

The several kinds of agar, of purified agars, of corresponding agar extracts, of paper extracts, and of cloth extracts were added to the basal medium in various proportions and combinations. In every experiment several portions of each type of medium were supplemented by the addition of small known amounts of thiamin.

At the end of one week observations were recorded on the growth of mycelium and appearance of sporangia. Dry weights of the mycelium in the flasks were determined as follows: Each flask was filled with hot distilled water and placed on a hot plate until the agar melted. The mycelium was then fished from the flask, washed in boiling distilled water, dried at 95–100° C. for 18–24 hours and weighed.[2]

THIAMIN CONTENT OF VARIOUS COMMERCIAL AGARS

In the basal culture medium growth of *Phycomyces* was lacking or negligible. The addition of thiamin to this medium resulted in abundant growth of *Phycomyces*.

Thiamin in Difco Granulated Agar. When the basal medium was solidified with Difco agar, lot 318721, in each test in which 0.5 or 1.0 g. agar

[2] A comparison of the dry weight of untreated mycelium of *Phycomyces* grown in liquid culture containing thiamin with the dry weight of mycelium which had been boiled and washed before drying showed the latter to be 12–15 per cent lower.

per flask was used, *Phycomyces* formed mycelium with sporangia in all four flasks (fig. 1). The dry weight was roughtly proportional to the amount of agar in the flask; for example, in experiment 16 (see table 1) the dry weight was 28.8 mg. with 1.0 g. agar, 13.9 mg. with 0.5 g. agar, 7.9 mg. with 0.25 g. agar, and 6.2 mg. with 0.125 g. agar. This growth of *Phycomyces* when agar was added to the basal medium indicated the presence in this agar of thiamin or its intermediates. From the relation between the amount of thiamin and the dry weight of *Phycomyces* grown in liquid culture it was calculated that the agar contained about 0.1 mμ mole thiamin per gram.

The most obvious differences in five tests of this agar were in experiments 10 and 11, in which the dry weights ranged from 19.0 mg. to 34.4 mg., or an indicated 0.07–0.16 mμ mole thiamin, per 1.0 g. agar. In one experiment, 29, with 1.0 g. and 0.5 g. agar the dry weights were 29.3 mg. and 17.5 mg. respectively for lot 318721, 24.9 mg. and 10.6 mg. for 325120, and 14.6 mg. and 8.8 mg. for 332408. In terms of equivalent amounts of thiamin the first two lots of agar contained about 0.1 mμ mole and the third lot less than one-half this amount. The variations in these results may be due to one or more of

TABLE 1

*Growth of Phycomyces Blakesleeanus on agars and on agar extracts. All cultures contained the same amounts of mineral salts, dextrose and asparagine. Dry weight is recorded in mg. per culture.**

Experiment number	Untreated agar		Purified agar		Agar extract from	
	1.0 g.	0.5 g.	1.0 g.	0.5 g.	1.0 g.	0.5 g.
			Difco granulated agar, Lot #318721			
10	19.0	12.2	0.5	1.3	. .	. . .
11	25.5	......	0.5	...	11.2	9.9
11	34.4	..	... ..	.		
13	28.4	16.7	.	0.2	12.1	7.6
16	28.8	13.9	0.2	0.2	.. ...	
16	27.9		1.0	.... .	. ..	
29	29.3	17.5	. .. .	... .	..	
Average	27.6	15.1	0.5	0.6	11.6	8.8
General average per 1.0 g.	28.9		0.9		14.6	
			Eimer and Amend flake agar			
11	2.2	.. ...	..		. ..	. .
12	3.9	3.2	1.5	1.8	. .....	
13	1.9	0.3	0.5	0.4	. ...	.. .
15	.. ...	....	.....	....	12.0	3.3
15	... ..	. ... .	....	.. ...	11.3	16.9
16	3.1	2.1	0.3	0.7	....	... .. .
16	3.5	..	0.8	. .. ..	. . ..	.. . .
Average	2.9	1.9	0.8	1.0	11.7	10.1
General average per 1.0 g.	3.4		1.4		16.0	

* Since the results presented here were confirmed by tests with flasks containing 0.25 g. and 0.125 g. agar, the latter data are not included in this table although they are occasionally cited in the body of the paper.

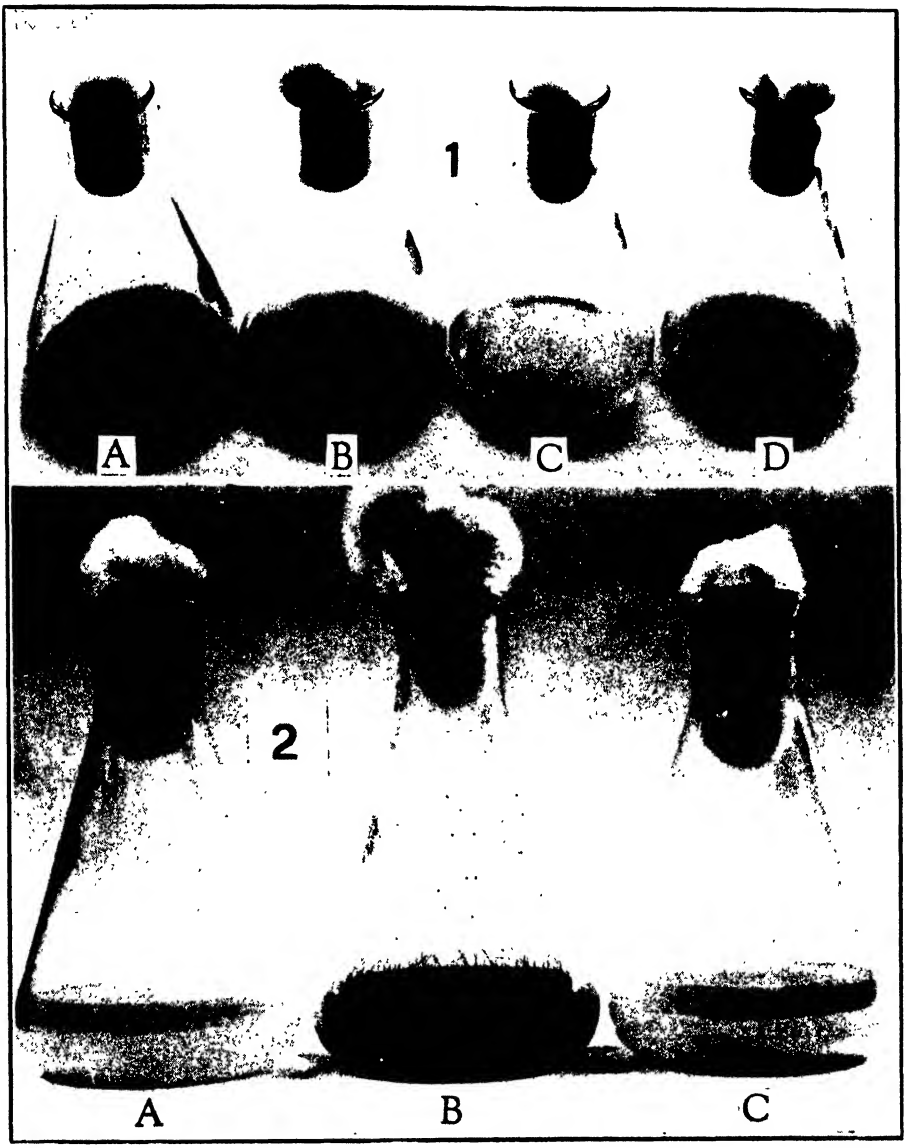

FIG. 1. Effect of different amounts of agar on growth of *Phycomyces*. Each flask contained 25 ml. of the same basal medium plus: A, 1.0 g. Difco agar; B, 0.5 g.; C, 0.125 g.; D, 0.125 g. agar and 0.1 mμ mole thiamin. Age 7 days. The darker appearance of the material in A (the flask on the left) is due to the larger amount of agar in the medium as well as to greater growth of mycelium with numerous sporangia. FIG. 2. Effect of different kinds of agar on growth of *Phycomyces*. Each flask contained 50 ml. of the same basal medium plus 1.0 g. agar; A, purified Difco agar; B, untreated Difco agar; C, untreated Eimer and Amend agar. Age 7 days. The darker appearance of the material in the middle flask is due to the substances in the untreated Difco agar as well as to the greater growth of mycelium with sporangia.

several causes, such as loss of mycelium in taking it from the flask, incomplete removal of the agar from the mycelium, unequal inoculation of the flasks in different experiments, or to some other factor. The lesser dry weight in the third lot of agar may indicate a difference in the amount of thiamin in separate lots of agar.

A known amount of thiamin added to a definite quantity of agar in the basal medium gave about 64 per cent of the dry weight that would be expected by adding that on liquid culture with added thiamin to that on the basal medium with agar; for example, addition to 1.0 g. agar of 0.05 mμ mole thiamin gave 31.4 mg. in place of the expected 45.0 mg., and of 0.5 mμ mole thiamin, 57.2 mg. in place of 100 mg. This may be due to some unexplained error, or it may indicate that the dry weight produced in an agar medium with added thiamin is less than that in a liquid medium with the same amount of thiamin. If this is true, the thiamin content of agar is more than is indicated by the figures mentioned, for all calculations of the probable amounts of thiamin were made on the relation between thiamin and dry weight of mycelium produced in liquid cultures.

Thiamin in Eimer and Amend flake agar. This agar produced sparse mycelium with few or no sporangia (fig. 2). Although greater dry weight usually was obtained in the presence of more agar the proportion was not definite, as it was with Difco agar; for example, in experiment 13 the resulting dry weights were 1.9 mg., 0.3 mg., 1.8 mg., and 1.2 mg. for 1.0 g., 0.5 g., 0.25 g., and 0.125 g. agar respectively. The very small dry weights average only 2.9 mg. with 1.0 g. agar (table 1), or less than 0.01 mμ mole thiamin. This amount was scarcely one-tenth of that found in Difco agar. Here, also, the addition of a known 0.5 mμ mole thiamin to 1.0 g. agar gave dry weight of about 60 per cent of what might have been expected.

Thiamin in crude, shred agar. This agar contained some thiamin. The dry weight was 5.3 mg. for 0.25 g. agar and 3.2 mg. with 0.125 g. agar, a response roughly proportional to the quantity of agar in the flask. The thiamin indicated was about 0.09 mμ mole in one gram of agar, an amount midway between those in the two other types of agar.

Because *Phycomyces Blakesleeanus* did not grow in a liquid medium but grew in the same medium when it was supplemented by a known quantity of agar, it would appear that commercial agar contains thiamin or its intermediates. Difco granulated agar contained more of this vitamin than did a crude, shred agar which in turn had more than the sample of Eimer and Amend flake agar used.

EFFECT OF THE PURIFICATION OF AGAR ON ITS THIAMIN CONTENT

Samples of Difco granulated agar and of Eimer and Amend flake agar were purified with pyridine and were then used to solidify the basal medium.

In all of the tests with purified agars *Phycomyces* formed only sparse mycelium and sporangia were generally absent or very rare (fig. 2). When thiamin was introduced into the medium with either of the purified agars, growth was prompt and definite. This indicated that the sparse growth of *Phycomyces* in the purified agar media was due to a lack of thiamin and not to toxicity of the purified agar caused by a residuum of pyridine or other causes.

With purified Difco agar, lot 318721, the dry weight of *Phycomyces* was never more than 3.6 mg. per 1.0 g. agar (table 1). The dry weight was not proportional to the amount of agar present; for example, in experiment 10 the dry weights were 0.5 mg., 1.3 mg., 0.9 mg., and 1.2 mg. with 1.0 g., 0.5 g., 0.25 g., and 0.125 g. agar respectively. This indicated considerably less than 0.01 mμ mole thiamin per 1.0 g. purified Difco agar, or less than one-tenth the amount present before the treatment with pyridine. The addition to 1.0 g. purified agar of 0.05 mμ mole thiamin gave 20 per cent of the anticipated dry weight and of 0.5 mμ mole gave 66 per cent of the expected weight. Added to 0.125 g. agar these same amounts of thiamin gave 30 per cent and 60 per cent, respectively, of the anticipated dry weights.

With purified Eimer and Amend agar the general average for the dry weight per gram of agar was 1.4 mg.; however individual determinations varied considerably (table 1). The dry weights were not proportional to the amount of purified agar; for example, in experiment 13 the dry weights were 0.5 mg., 0.4 mg., 2.3 mg., and 1.3 mg. with 1.0 g., 0.5 g., 0.25 g., and 0.125 g. agar respectively. This was equivalent to somewhat less than 0.01 mμ mole thiamin in 1.0 g. purified Eimer and Amend agar, possibly one-half the amount present before extraction. The addition of 0.5 mμ mole thiamin to 0.5 g. purified Eimer and Amend agar gave dry weight of 31.7 mg. in place of the calculated 84.0 mg., to 0.125 g. agar, 3.3 mg. in place of 19.0 mg.

Crude shred agar subjected to electrodialysis formed scanty mycelium with dry weights of 2.6 mg. and 1.0 mg. per 0.25 g. and 0.125 g. agar respectively. The relation between the dry weight of *Phycomyces* and the amount of agar was closer than it was for either of the two types of agar purified with pyridine. The amount of thiamin indicated was approximately 0.02 mμ mole, or about one-fourth the amount present in the shred agar before electrodialysis and about twice as much as in the two agars purified by pyridine.

From these results it would appear that thiamin was largely or completely removed from agar by leaching with pyridine. Electrodialysis also removed thiamin from agar but was somewhat less effective than leaching with pyridine. Several tests, not reported here in detail, indicated that washing agar in distilled water removed some thiamin, but further study is needed to determine whether leaching with water is as satisfactory as with pyridine.

THIAMIN IN AGAR EXTRACTS

The agar extracts were added to the basal medium in amounts equivalent to the quantities of agar used in the earlier tests. Growth of *Phycomyces* on each of these agar extracts was superior to that on either of the purified agars.

The dry weights with Difco agar extract averaged 11.6 mg. for the extract of 1.0 g. agar and 8.8 mg. for 0.5 g. agar (table 1). The general average for the equivalent of 1.0 g. agar was 14.6 mg., or about 0.04 mμ mole thiamin, not quite one-half the amount in this same lot of untreated agar.

With Eimer and Amend agar the dry weights averaged 11.7 mg. and 10.1 mg. for the extract of 1.0 g. and 0.5 g. agar respectively (table 1). The general average for the equivalent of 1.0 g. agar was 16.0 mg., or 0.05 mμ mole thiamin, which was about five times as much as was found in the original Eimer and Amend agar.

These differences between the thiamin content of the extract and the original agar are difficult to understand. Aside from the possibility of experimental error, which I do not believe is responsible, the following possibilities should be considered: Some thiamin may have been destroyed in the process of concentrating the agar extract under alkaline conditions created by the presence of pyridine. This would account for the smaller amount of thiamin found in the extract of the Difco agar as compared to the original agar. The more rapid percolation of the aqueous pyridine through the Eimer and Amend flake agar allowed periods when little liquid was present and the agar mass was moist and well aerated. This may have permitted the development of micro-organisms which increased the thiamin content of the extract beyond that of the original agar. The validity of these suggestions has not been determined, but they are offered as possible explanations.

When Difco agar extract was added to an equivalent amount of purified Difco agar in the basal medium, this combination gave dry weights of 2.8 mg., 2.0 mg., 2.2 mg., and 2.0 mg. for 1.0 g., 0.5 g., 0.25 g., and 0.125 g. respectively. With Eimer and Amend agar a similar combination of purified agar plus agar extract gave 1.9 mg., 0.7 mg., and 1.3 mg. for 0.5 g., 0.25 g., and 0.125 g. respectively. A comparison of these figures with those noted earlier shows that growth of *Phycomyces* was much less here than on the untreated agar, was less than on the agar extract, was less than on purified agar with 0.05–0.5 mμ mole added thiamin, and was little more than on the purified agar; this was true for both the Difco and the Eimer and Amend agars and their extracts. These results confirmed and emphasized the point suggested in the earlier data that changes may have taken place during the process of leaching the agar, changes which were more than the simple washing out

of thiamin. Whatever the causes may be, it is obvious that purified agar plus agar extract is not equivalent to the original, untreated, commercial agar.

WATER EXTRACTS FROM FILTER PAPER AND CLOTH

Growth of *Phycomyces* with extracts from filter paper indicated less than 0.01 mμ mole thiamin per 1.0 g. Eaton and Dikeman paper and almost 0.02 mμ mole thiamin for 1.0 g. Whatman paper. Similar tests indicated about 0.03 mμ mole thiamin per 1.0 g. cheesecloth and about 0.15 mμ mole thiamin per 1.0 g. tobacco cloth. While these amounts of thiamin in filter paper and cheesecloth may seem small, especially those from the filter paper, they are definite and are large enough to cause a serious experimental error if the presence of thiamin were not realized.

DISCUSSION

Although a high degree of accuracy cannot be claimed for experiments in which the growth substances occur in such minute amounts as they did in the materials which were used, demonstration of a measurable quantity of thiamin[3] in commercial agar, in cheesecloth, and in filter paper emphasizes the need for care in laboratory procedure in which growth substances are involved. Proper precautions are especially important, because growth substances are active in minute amounts such as may be present in agar or obtained from filter paper or cheesecloth used in the laboratory.

Purification of agar with five per cent aqueous pyridine appeared satisfactory, but other methods, such as washing with water, may prove as effective and simpler for the removal of thiamin.

The dry weight of *Phycomyces* on agar media to which thiamin was added and on purified agar to which agar extract was added was less than anticipated from growth of this organism in liquid cultures in the presence of known quantities of thiamin. These observations raise the question whether the response of *Phycomyces* to a given quantity of thiamin may not be greater in a liquid medium than in an agar medium because of lessened availability of thiamin in the presence of agar, because of decreased supply of water or another essential substance, or because of some other factor.

SUMMARY

1. *Phycomyces Blakesleeanus* grew better and produced greater dry weight in a medium solidified with Difco agar or with Eimer and Amend agar than it did in the same medium without the agar. Because the basal medium contained minerals, sugar and asparagine but no added thiamin, this result was interpreted as indicating the presence of thiamin or its

[3] It should be pointed out that this study is concerned not only with thiamin as such but also with the thiazole and pyrimidine intermediates to which also *Phycomyces* responds.

intermediates in these agars. Different kinds of agar varied in thiamin content.

2. Thiamin was largely or completely removed by leaching the agar with five per cent aqueous pyridine, as was shown by lack of growth of *Phycomyces* in purified agars and by its growth in the agar extracts. Electrodialysis proved no more efficient in purifying agar than washing with five per cent pyridine.

3. Cheesecloth, tobacco cloth and filter paper also were found to contain appreciable quantities of thiamin as measured by the growth of *Phycomyces.*

4. Washing agar, filter paper and cheesecloth with distilled water removed some of the thiamin.

5. Demonstration of thiamin in these frequently used materials emphasized the need for care in laboratory technique.

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THE EFFECT OF ACTINOMYCES ALBUS AND OF THIAMIN ON THE GROWTH OF TRICHOPHYTON DISCOIDES

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INTRODUCTION

The fungus *Trichophyton discoides* Sabouraud is a common cause of tinea in calves in Uruguay. It is also a frequent cause of tinea of the scalp, the beard, and the glabrous skin of man. These infections are usually very inflammatory. On Sabouraud's media, *T. discoides* grows slowly and produces a flat, smooth, and usually glabrous giant colony; some strains produce a central umbo and short velvet; the colony has a brownish neutral color. Sabouraud (5) did not describe conidia; but in 1934 Lebasque (2) observed aleurospora and closterospora in cultures on different cereals and on horse dung. Cazalbou (1) describes phases in the cultures of *T. discoides;* where *T. discoides* is mentioned in this paper the so-called normal phase is to be understood.

During study of our strain 688, a culture on glucose-peptone agar was accidentally contaminated by an *Actinomyces* of the *Actinomyces albus* group. *A. albus* (strain 693) began to grow at a distance of four cm. from a colony of *T. discoides*. After 15 days the sector of the colony of *T. discoides* nearest to the colony of *A. albus* became folded and velvety while the other sectors remained smooth and glabrous. Folding of the colony was due to an increase of the growth. The fact was confirmed many times (fig. 1). If the agar between the two colonies was cut, the *Actinomyces* had no further influence over the growth of the *Trichophyton*.

The same results were obtained with another strain of the *Actinomyces albus* group, isolated from the soil (strain 800). A successful attempt was made also to increase the growth of another strain of *T. discoides* (strain 461).

These experiments proved that our strains of *Actinomyces albus* have a beneficial effect on the growth of *T. discoides* and that this effect is produced by a water-soluble substance. The importance of thiamin for plant growth has been recently emphasized by Robbins (3). In the thought that perhaps thiamin was the cause of the increase in the growth of *T. discoides,* the following experiments were made.

EXPERIMENTS WITH GLUCOSE-PEPTONE AGAR

The culture medium was made up as follows: bacto-peptone 10 g., pure Grübler glucose 40 g., agar 20 g., distilled water 1 l. Approximately 100

ml. of this medium was poured in flasks with a flat side 19 cm. long and 5.5 cm. wide. Six flasks were arranged in three groups of two each. The thiamin chloride used was the synthetic crystalline product of Roche called "Aneurine."

To the flasks of the first group nothing was added. To each flask of the second group the mycelium of three cultures of *A. albus,* also on glucose-peptone agar, was added; these cultures had been incubated 15 days at 37° C. To the flasks of the third group 0.5 ml. of a 1:10,000 thiamin solution was added. The six flasks were heated at 100° C. for 30 minutes and then placed on their sides until the medium solidified. The layer of agar was 1 cm. thick.

The six flasks were inoculated with *T. discoides* in order to obtain giant colonies, and kept for 20 days in the incubator at 37° C. After this period the colonies which were growing on glucose-peptone agar (first group of flasks) were flat, smooth, and glabrous; they had produced a scanty mycelium and were from 4.5 to 5 cm. in diameter (fig. 2). The colonies in the flasks of the second group, with mycelium of *A. albus,* were folded and covered with a short velvet; they had produced abundant mycelial growth (fig. 3). The colonies in the flasks of the third group, with thiamin, were even more folded and velvety; they were from 3.5 to 4.5 cm. in diameter (fig. 4).

Aleurospora and closterospora were produced by the colonies in the flasks with mycelium of *A. albus* or thiamin, but were very scarce. *T. discoides,* when cultivated in media with thiamin or mycelium of *A. albus,* grows only over the surface of the agar; but when cultivated on media to which thiamin or *A. albus* had not been added, it produces a scanty growth over the surface and also submersed mycelium.

I inoculated peptone-glucose agar with mycelium of the folded, velvety colonies, which were growing in the medium with thiamin, and obtained again flat, smooth, glabrous colonies.

These experiments demonstrate that *Actinomyces albus* elaborates a thermostable and water-soluble substance which has an effect on the growth of *T. discoides* similar to that of thiamin.

EXPERIMENTS WITH SYNTHETIC MEDIA

The following experiments were undertaken to determine if *Actinomyces albus* is really autotrophic for thiamin. The researches of Schopfer and of Robbins have demonstrated that *Phycomyces Blakesleeanus* Burgeff requires thiamin and that it is unable to synthesize this growth substance from sugar, asparagine, and minerals; this fungus produces a mycelial mat and sporangiophores only when supplied with thiamin or with both its intermediates, thiazole and pyrimidine.

P. Blakesleeanus was grown in a medium similar to that used by Robbins


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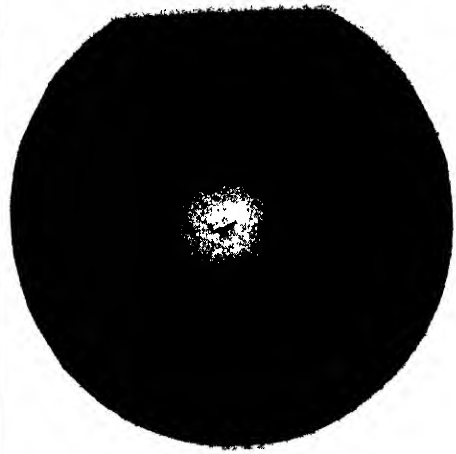
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Fig. 1. *Actinomyces albus* (above) and *Trichophyton discoides* (below) growing in the same flask. *A. albus* benefits the growth of the upper part of the colony of *T. discoides*. Fig. 2. *Trichophyton discoides* (strain 688). Giant colony aged 20 days growing on glucose bacto-peptone agar. ×1. Fig. 3. *Trichophyton discoides* (strain 688). Giant colony aged 20 days growing on glucose bacto-peptone agar plus mycelium of *Actinomyces albus*. ×1. Fig. 4. *Trichophyton discoides* (strain 688). Giant colony aged 20 days growing on glucose bacto-peptone agar plus thiamin. ×1.

(4), its composition being: dextrose 100 g., asparagine 1.0 g., $MgSO_4 \cdot 7H_2O$ 0.5 g., KH_2PO_4 1.5 g., bacto-agar 10 g., redistilled water 1 l. To this medium 0.1 ml. of the following solution of mineral supplements was added: H_3BO_3 5.7 mg., $CuSO_4 \cdot 5H_2O$ 18.6 mg., $(NH_4)_2Fe_2(SO_4)_4 \cdot 24H_2O$ 173 mg., $MnCl_2 \cdot 4H_2O$ 7.1 mg., ammonium molybdate (85 per cent) 3.6 mg., $ZnSO_4 \cdot 7H_2O$ 79 mg., redistilled water 100 ml. The medium was poured in 250 ml. Erlenmeyer flasks of Murano glass, 40 ml. in each flask. The flasks were arranged in three groups.

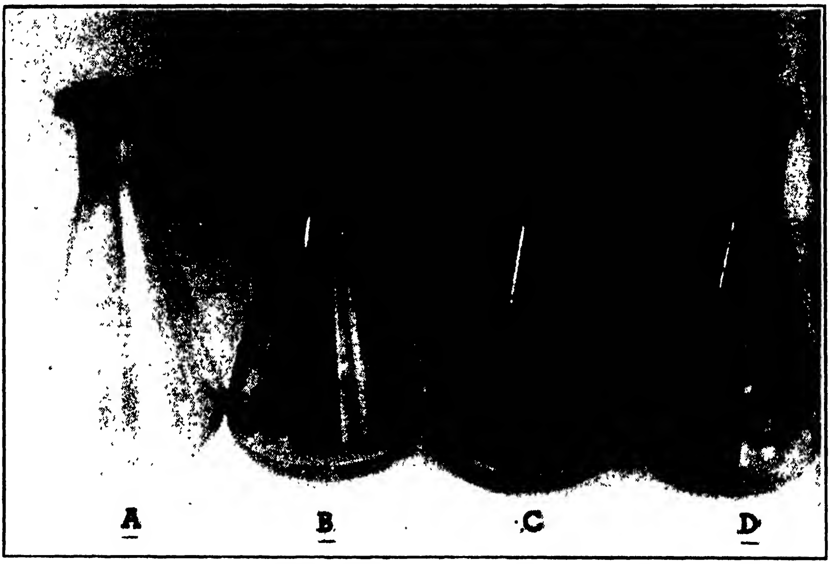

FIG. 5. Elaboration of thiamin by our strain 800 of the *Actinomyces albus* group tested by the growth of *Phycomyces Blakesleeanus*. From left to right: A. Culture on a medium lacking thiamin; no sporangiophores. B. On the same medium plus filtered cultures of *Actinomyces albus;* few sporangiophores. C. On the same medium plus mycelium of *A. albus;* luxuriant growth constituted mainly by a huge number of sporangiophores. D. On the same medium plus thiamin.

Group 1. To two flasks were added 10 ml. of Czapek medium of the following composition: $NaNO_3$ 2 g., K_2HPO_4 1.0 g., $MgSO_4 \cdot 7H_2O$ 0.5 g., KCl 0.5 g., $FeSO_4 \cdot 7H_2O$ 0.01 g., dextrose 40 g., bacto-agar 20 g., redistilled water 1 l. The media were melted and then mixed. After solidification of the medium the two flasks were inoculated with mycelium of *Phycomyces Blakesleeanus,* the plus strain. After 10 days at a temperature between 15° and 20° C., a scanty, sparse mycelial growth was evident; no sporangiophores were produced (fig. 5 A).

Group 2. To two flasks were added 10 ml. of Czapek medium and four drops of 1:10,000 thiamin solution. *P. Blakesleeanus* developed fairly well; long sporangiophores and a mycelial mat were produced (fig. 5 D).

Group 3. To four flasks were added 10 ml. of cultures of *Actinomyces albus* (strain 800) on Czapek medium. These cultures had been incubated at 37° C. for 15 days. After it had been heated at 100° C., *A. albus* died. *P. Blakesleeanus* grew in these flasks as well as in the flasks with thiamin (fig. 5 C).

A. albus was grown also in Czapek liquid medium. After 15 days the culture was filtered through a Berkefeld filter and the extract was added to the agar which lacked thiamin. In this medium only a few sporangiophores were produced (fig. 5 B).

The experiments demonstrate that our strain 800, of the *A. albus* group, is autotrophic for thiamin.

OTHER EXPERIMENTS

Growth of Phycomyces Blakesleeanus on glucose-peptone agar: This fungus grew well on this medium; but the addition of from 0.00005 to 0.0001 g. thiamin to 100 ml. of the medium produced a great increase in growth. This experiment seems to show that our medium (Sabouraud's formula) has an insufficient quantity of thiamin or of one or both intermediates.

Cultures of Trichophyton discoides on synthetic media: I was unable to cultivate *T. discoides* on the two media referred to above. The addition of thiamin to such media did not modify this result.

An inhibitory effect of A. albus over P. Blakesleeanus: Glucose peptone agar was inoculated with our strain 693 of *A. albus* in order to have a few colonies. Six days later we inoculated the same flasks, where *A. albus* was already growing, with mycelium of *P. Blakesleeanus.* We observed that the growth of *Phycomyces* was increased near the colonies of *A. albus;* but very close to the colonies (1 mm.) the mycelium of the *Phycomyces* ceased growth.

DISCUSSION

The above experiments indicate that our strains of *Actinomyces albus* are autotrophic for thiamin, since their mycelium permits *Phycomyces Blakesleeanus* to produce a luxuriant growth and long sporangiophores in media which lacked thiamin. No information was furnished of interest in the physiology of *Trichophyton discoides;* our experiments only demonstrate that thiamin benefits its growth on media prepared with bacto-peptone.

In medical mycology the identification of species is frequently made by the aspect of the giant colonies. The colony growth of *T. discoides* may vary greatly with the addition of small amounts of thiamin. The culture media are not always prepared with the same products, and one must therefore be cautious not to attach too much importance to small differences between the descriptions and figures of different authors. Many such small differences in

the aspect of the giant colonies have been considered as deserving specific rank.

SUMMARY

Strains of actinomyces, of the *Actinomyces albus* group, have a stimulating influence over the growth of *Trichophyton discoides* Sabouraud, when the latter is grown on glucose bacto-peptone agar. The giant colonies, usually flat, smooth, and glabrous, become folded and velvety.

The addition of thiamin to the glucose bacto-peptone agar has the same effect as *A. albus* on the growth of *T. discoides*.

An agar medium lacking thiamin to which cultures of *A. albus* on glucose Czapek medium have been added permits *Phycomyces Blakesleeanus* to produce long sporangiophores as well as if thiamin had been added. Filtered cultures of *Actinomyces albus* in liquid Czapek medium have a similar but less marked effect.

The author is deeply indebted to William J. Robbins, Director of The New York Botanical Garden, for his advice and for the strain of *Phycomyces Blakesleeanus* that he kindly furnished us.

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STUDIES IN THE CRASSULACEAE—III. SEDUM, SUBGENUS GORMANIA, SECTION EUGORMANIA[1]

ROBERT T. CLAUSEN

Generic limits in the Crassulaceae are indefinite. Probably there never will be agreement regarding the boundaries of the various genera. The group of species which Britton (1903) included in *Gormania* was originally characterized by the horizontal rootstocks, the petals united below the middle and somewhat spreading above, the stamens borne on the corolla and the carpels united below. Yet, if one surveys the genus *Sedum*, even denuded of the many generic segregates, there still remain species which possess one or the other of all characters indicated as diagnostic for *Gormania*. *S. spathulifolium* has the horizontal rootstocks, *S. adolphii* and *S. alsinefolium*, the petals united below the middle and the stamens borne on the corolla, and *S. fusiforme* the carpels united below. None of these characters is an absolute criterion for *Gormania*. Further, the statement about the petals must be modified somewhat. Study of fresh flowers indicates that there is considerable variation in the degree of fusion of the petals. They are only weakly united and can be pulled apart along the lines of fusion. Some of the species, referred by Britton to *Gormania*, now require somewhat different treatment. These include *Gormania debilis*, *G. oregana* and *G. anomala*. All three are morphologically intermediate between *Gormania* and *Sedum* in the strict sense. Cytological evidence indicates that the first is far removed from *Gormania*, while the floral structure of the last two divorces them from *Gormania* proper, but places them in related sections.

Cytological studies by Dr. Hollingshead (1942) suggest relationship of *Gormania* with the ***Rosulata*** of *Sedum*, also perhaps with the genus ***Echeveria*** which has been sampled by Baldwin (1939). The chromosomes are exceedingly small, elliptical or almost round, and rather numerous, either 30 or 90 in the diploid condition. These are illustrated in this issue in the paper by Hollingshead (1942). *S. debile*, treated as a *Gormania* by Britton, is $2n = 14–18$. Its chromosomes are larger and markedly 2-armed, quite different in shape from those of any species of *Gormania*. Cytologically it is closer to *S. divergens* ($2n =$ *circa* 16) which has two-armed chromosomes. *Sedum spathulifolium* of the section ***Rosulata***, generally acknowledged as properly a

[1] This investigation was carried on with the aid of a grant from the Penrose Fund of the American Philosophical Society and a grant from the Trustee-Faculty Committee on Research of Cornell University. The cytological data are the result of studies by Dr. Lillian Hollingshead.

part of *Sedum,* is cytologically similar to *Gormania.* The chromosomes are small and round and, in all plants studied, the diploid number is 30. Although the cytological evidence indicates that section *Rosulata* and *Gormania* are closely similar, the differences in floral structure, inflorescence and leaves are considerable. In *Rosulata* the petals are not connivent, but are more quickly spreading in rotate fashion; the inflorescence is a three-parted cyme, not a paniculate cyme; and the leaves of the rosettes are flatter and less leathery. On a basis of these three criteria, *Gormania anomala* Britton is clearly a member of the section *Rosulata.* Further, it is not specifically different from *S. spathulifolium.*

Sedum oreganum merits special attention. Although originally placed in *Gormania* by Britton, it differs markedly in certain details. The petals are narrowly lanceolate, long acuminate, and connivent for only one-eighth of their length. The sterile rosettes are on leafy stems, with the leaves not leathery after the fashion of *Gormania.* Instead, in texture and appearance, the leaves are more like those of *S. divergens.* Probably, *S. oreganum* belongs in a section by itself, Section **Oreganica,** n. sect. (sectio nov., petalis anguste lanceolatis, longe acuminatis, erectis sed paululum conniventibus; rosulis caulibus 2–10 cm. longis, foliis carnosis sed non coriaceis).

In order to show seeming natural relationships, *Gormania* may now be treated as a subgenus of *Sedum* comprising three sections. The first of these sections, here designated as *Eugormania,* in the union of the petals below, seems to be morphologically closest to *Echeveria,* while the other two sections are morphologically closer to *Eusedum.*

Subgenus **Gormania** (Britton) Clausen, stat. nov.

Gormania Britton, Bull. N. Y. Bot. Gard. **3**: 29. 1903. Named in honor of M. W. Gorman of Portland, Oregon. TYPE SPECIES: *Cotyledon oregonensis* Watson.

Characteristics of this subgenus are the horizontal rootstocks, the prominent sterile rosettes and the petals which are erect at base for 1/10 or more of their length. The chromosomes are small and more or less round, in a euploid series of $n = 15$.

KEY TO THE SECTIONS OF GORMANIA

A. Petals connivent or united for $\frac{1}{4}$ or more of length, divergent above; sterile rosettes with the leaves thick and leathery, usually glaucous; inflorescence a paniculate cyme .... 1. Section *Eugormania*

AA. Petals free from the base or united for $\frac{1}{8}$ of length, erect or widely spreading above; leaves fleshy, but not leathery; inflorescence a 3-parted cyme, sometimes compound.

B. Petals erect at base for about $\frac{1}{10}$ of length, then widely spreading; leaves green or gray, often glaucous .... 2. Section *Rosulata*

BB. Petals erect throughout, united at base for ⅛ of length; leaves very fleshy, green suffused with red, never glaucous .... 3. Section *Oreganica*

Section Eugormania

This section is distinguished by the characters indicated in the above key. The type species is the same as for the subgenus.

In the discussion under the species and subspecies, names of herbaria are abbreviated as: Bailey Hortorium, Cornell University, BH; Department of Botany, Cornell University, CU; California Academy of Sciences, CAS; Gray Herbarium, Harvard University, GH; New York Botanical Garden, NY; Santa Barbara Museum, SBM; Stanford University, DS; U. S. National Herbarium, US; and Willamette University, WILLU. Besides the specimens in the herbaria listed above, I have also made a rapid survey of the material in the herbarium of the University of California at Berkeley.

KEY TO THE SPECIES OF EUGORMANIA

A. Inflorescence and upper part of stem glandular pubescent, strongly reflexed before flowering time; leaves of rosettes glandular-ciliate; petals yellow .... 2. *Sedum glanduliferum*

AA. Inflorescence and upper part of stem glabrous, usually erect before flowering time; leaves of rosettes usually not ciliate; petals yellow, white or pink .... B

B. Flowers yellow or pale yellow, sometimes fading to white or pink in age; inflorescence a paniculate cyme; leaves of rosettes 1–3 cm. long .... 1. *Sedum obtusatum*

BB. Flowers white, creamy white or pink; inflorescence a dense paniculate or corymbose cyme; leaves of rosettes 1–4.5 cm. long.

C. Flowers white or creamy white; sepals ovate, 2–3 mm. long. 3. *Sedum oregonense*

CC. Flowers pink or pale pink, rarely white; sepals lanceolate or ovate, 2–5 mm. long .... 4. *Sedum laxum*

1. Sedum obtusatum A. Gray.

From the other species of the Section *Eugormania, Sedum obtusatum* may be distinguished by the yellow flowers which are borne in a glabrous paniculate cyme. The species is nearest to *S. oregonense,* from which it is only doubtfully distinct in the northern part of its range. That species has creamy white flowers, a paniculate-cymose inflorescence which is broadest and densest above, and larger rosette leaves; also, so far as present investigations indicate, it is hexaploid.

Sedum obtusatum may be divided into a southern subspecies with small rosette leaves and bright red stems and a northern subspecies with larger leaves and stems which are only slightly red or pink. The southern element, including the type, is native in the Sierra Nevada, ranging north to the region of the Feather River (fig. 1). The other race has its center of distribution in the southern Cascade Mountains. The two subspecies are separated in the following key:

A. Basal leaves relatively small, 0.5–2.5 cm. long, usually broadly rounded or truncate at apex; stems of rosettes bright red .... 1a. *S. obtusatum* ssp. *typicum*
AA. Basal leaves usually larger, 1–3.0 cm. long, usually retuse at apex; stems of rosettes pale red or pink .... 1b. *S. obtusatum* ssp. *boreale*

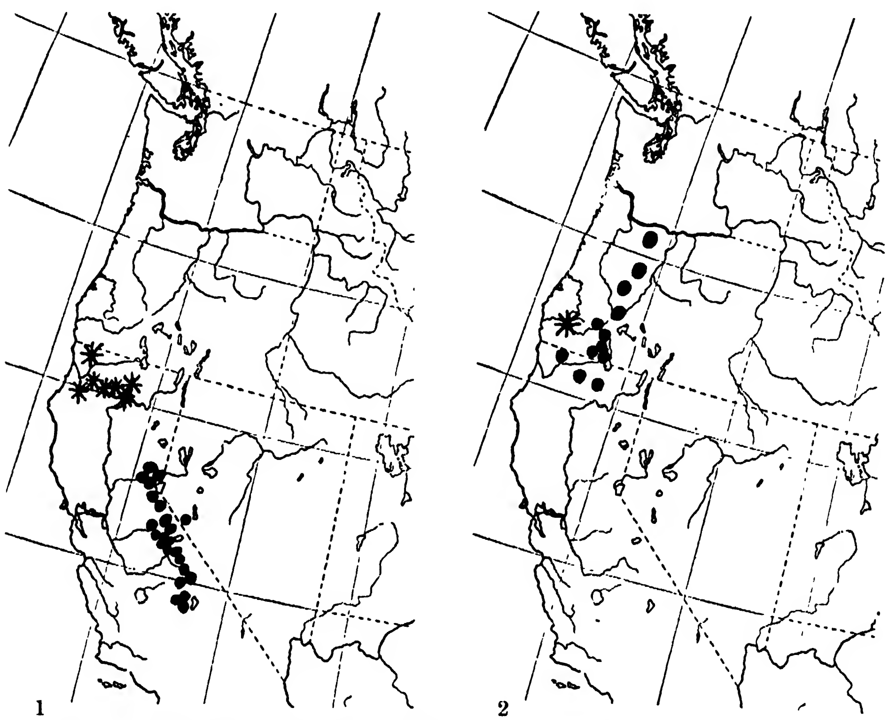

FIG. 1. Distribution of *Sedum obtusatum*, ssp. *typicum* (dots) and ssp. *boreale* (stars). FIG. 2. Distribution of *Sedum oregonense* (dots) and *S. glanduliferum* (star).

1a. SEDUM OBTUSATUM A. Gray, ssp. TYPICUM[2]

Sedum obtusatum A. Gray, Proc. Am. Acad. 7: 342. 1868. TYPE specimens are in Gray Herbarium. Type sheet bears four collections from an equal number of localities: Vernal Falls; Yosemite Valley; Mt. Hoffman; and a peak in the Sierra Nevada. The specimen from Yosemite Valley, collected by J. Torrey, 1865, no. 143, is fragmentary and perhaps referable to *S. spathulifolium*. The other three specimens seem to be closely similar to each other and agree with Gray's original diagnosis. I have visited and collected plants at both Vernal Falls and Mt. Hoffman.

[2] Regarding the use of the epithet *typicus*, the following quotation from Gentes Herbarum (4[8]: 292. 1940) will explain my usage in this paper and elsewhere. "As employed by the writer, following Article 30, Recommendation 18 of the International Rules of Botanical Nomenclature, the epithet var. *typica* is not regarded as a new name, but as a parenthetical practical device to permit accurate designation of the typical element of a species. It has no nomenclatorial standing and does not require the citation of an authority. Whenever a variety or a subspecies is described or placed in a species, the typical element of that species automatically becomes variety or subspecies typica."

Gormania obtusata (A. Gray) Britton, Bull. N. Y. Bot. Gard. **3**: 29. 1903.

Gormania Hallii Britton, Bull. N. Y. Bot. Gard. **3**: 29. 1903. TYPE specimen, at the New York Botanical Garden, is from the vicinity of Tuolumne Meadows, in the Canadian Zone, at 8500–9500 ft. alt., Yosemite National Park, Calif., July, 1902, *H. M. Hall and E. B. Babcock 3545.* I have collected specimens on Lembert Dome, the type locality, at the eastern end of Tuolumne Meadows. These plants closely match my collections from Mt. Hoffman, and except for their later development, which is to be expected because of the higher altitude, are fair matches for plants from the region of Yosemite Valley. The shape of the sepals is not sufficiently different or reliable to distinguish this population.

Gormania Burnhami Britton, Bull. N. Y. Bot. Gard. **3**: 30. 1903. TYPE specimen, at the New York Botanical Garden, is from along the trail between Lake Eleanor and Lake Vernon, Tuolumne Co., Calif., July 16, 1894, *S. H. Burnham.* I have collected plants at the type locality. As the flowers mature, the petals fade to pink, as is the condition in plants in the Yosemite Valley. I find no basis to segregate this from the common population of the Sierra Nevada.

Cotyledon obtusata (A. Gr.) Fedde, Just's Bot. Jahresbericht **31** (1): 827. 1904.

Cotyledon Burnhamii (Britt.) Fedde, ibid.

Cotyledon yosemitensis Fedde, ibid. Based on *Gormania Hallii* Britton.

Echeveria obtusata (Gray) Nels. & Macbr., Bot. Gaz. **56**: 476. 1913.

Echeveria Brittonii Nels. & Macbr., Bot. Gaz. **56**: 476. 1913. Based on *Gormania Hallii* Britton.

Sedum rubroglaucum Praeger, Jour. Bot. **57**: 51. 1919. Described from plants sent by H. M. Hall, originally collected along the Short Trail, Yosemite Valley. I see no reason for distinguishing this from *S. obtusatum.* The crimson stem and glaucous leaves are both characteristic of the species, while the larger flower-size is not significant.

Sedum obtusatum var. *Hallii* (Britton) Smiley, Univ. Calif. Pub. Bot. **9**: 213. 1921.

Sedum Hallii (Britton) Praeger, Jour. Roy. Hort. Soc. **46**: 241. 1921.

Sedum Burhamii (Britton) Berger *in* Engl. & Prantl, Nat. Pflanzenfam. ed. 2, **18a**: 451. 1930.

Echeveria Hallii Berger, pro synon., ibid. 458.

Perennial with rootstocks to 5 mm. thick, bearing rosettes of thick, fleshy, spatulate leaves, obtuse or rounded at apex, glaucous, usually suffused with red or pink, 0.5–2.5 cm. long and 0.2–0.7 cm. wide; spreading by the production of numerous offsets, of which the stems are red; floral shoots erect, 3–16 cm. high, glabrous throughout, often suffused with red; cauline leaves alternate, oblong-spatulate, spurred, reduced upwards; inflorescence a paniculate cyme, 1.5–11 cm. long and 1.5–5 cm. broad; floral bracts spatulate to linear-oblong; sepals ovate-lanceolate, obtuse, erect, green, suffused with pink in age, 2 to 4 mm. long, united for 1 mm. at base; petals erect below and connivent for 2/5 their length, somewhat spreading above and keeled dorsally, oblong-lanceolate, 6–9 mm. long, "lemon yellow" when fresh, fading to buff or pinkish in age; stamens 4–6 mm. long, anthers yellow, filaments pale yellow;

nectar-scales transversely linear, about 0.8 mm. wide and 0.2 mm. long; carpels 6–7 mm. long, erect, greenish yellow to pinkish, becoming red in fruit.

The distribution of ssp. *typicum* is in the Sierra Nevada from Kaweah Peaks, Tulare Co., Calif., north to the headwaters of the Feather River, Plumas Co., and east to Aurora, Nevada. The altitudinal distribution is from 5000 to 13,000 ft. Habitat is usually on exposed rocks, in crevices and among boulders, occasionally in woods on rocky slopes.

Specimens seen: Highest altitude—11,000–13,000 ft., Lake of Islands, Kaweah Peaks, Tulare Co., Calif., *W. R. Dudley 2394* (DS); lowest altitude —5000 ft., Nevada Falls, Yosemite National Park, *R. T. Clausen & H. Trapido 4801* (BH, CU); northernmost—Feather River Region, Lake Center Camp, Plumas Co., Calif., *Anna Head* (CAS); easternmost—Aurora, Mineral Co., Nevada, *Mrs. J. D. Wright* (SBM); westernmost—Emigrant Gap, Placer Co., Calif., *Mrs. C. E. Miller* (CAS); southernmost—same as highest altitude; oldest—1860–62, Mt. Hoffman, *W. H. Brewer 1678* (GH). Number of collections seen—136.

The flowering time is from June to July; the fruiting time from July to August. Depending on the altitude and exposure, the leaves vary in size and in redness, also the flowers vary somewhat in size and in number in the inflorescence, but these fluctuations seem unworthy of nomenclatorial recognition. Specimens from Donner Lake, *A. A. Heller 7105* (NY, US) were given a manuscript name by J. N. Rose, but I do not regard them as sufficiently different to deserve taxonomic recognition.

A plant from Nevada Falls (*R. T. C. 4801*) and another from Lake Vernon (4810), both in Yosemite National Park, have a $2n$ number of 30. Another specimen from the slope of Mt. Hoffman (*R. T. C. 4823*) has an n number of 15. Since genetical work has not been carried on in the present investigation, there is no information regarding the compatibility of *S. obtusatum* with *S. oregonense* and the various subspecies of *S. laxum*, its nearest allies. Should these be freely interfertile, they ought all to be treated as subspecies of *S. obtusatum*. Each of these populations, here treated as species on a basis of morphological differences, occupies a distinct range.

S. obtusatum is relatively common in the horticultural trade, where it is generally correctly listed. The plant requires good drainage and much sunlight.

1b. Sedum obtusatum ssp. **boreale** Clausen, ssp. nov.—Planta foliis majoribus quam ssp. *typicum*, 1–3 cm. longis, plerumque retusis ad apicem; caules rosellarum roseatae.

Flowering plants relatively larger than ssp. *typicum*, 8–20 cm. high, rosette leaves spatulate, usually retuse at apex, 1–3 cm. long and 0.4–1.3 cm. wide, somewhat glaucous, occasionally reddish on the margins; stems of the offsets pale red or pink; floral shoots erect, glabrous, often suffused with pink; cauline leaves oblong-spatulate, spurred, reduced upwards; inflorescence an elongate paniculate cyme, 2–12 cm. long and 1.5–5.0 cm. wide; floral bracts spatulate to narrowly linear; sepals ovate, acute, 2–3 mm. long, united at base, green; petals erect below and connivent for 2/5–3/5 their length, somewhat spreading above, oblong-lanceolate, 6–7 mm. long,

pale to deep yellow, becoming white or pink with age; stamens 5–6 mm. long; carpels 6–7 mm. long. Type in the herbarium of the Department of Botany, Cornell University, from rocky slope, east side of Mud Creek Canyon, Mt. Shasta, Calif., July 26, 1940, *R. T. Clausen and H. Trapido 4952;* isotypes in the Bailey Hortorium and elsewhere.

Ssp. *boreale* occurs at altitudes from 4000–7500 ft. in the Southern Cascades and Coast Ranges of northern California.

Specimens seen: Highset altitude—6500–7500 ft., Caribou Basin, Salmon-Trinity Alps, Siskiyou Co., Calif., *J. T. Howell 13450* (CAS); lowest altitude—4000 ft., Bear Wallows, trail to Preston Peak, Del Norte Co., *Doris K. Kildale 8800* (DS); northernmost—Little Grayback, Siskiyou-Del Norte County Line, *Edward Lee* (DS); easternmost—east side of Mud Creek Canyon, Mt. Shasta, Siskiyou Co., *R. T. C. 4952* (BH, CU); westernmost—same as lowest altitude; southernmost—same as highest altitude; oldest—June 15–30, 1897, Mt. Shasta, Siskiyou Co., *H. E. Brown 440* (DS); regarding this collection, there is reason to doubt whether the plants were collected on Mt. Shasta or elsewhere. Number of collections seen—21.

The flowering time of ssp. *boreale* extends from late May to July, depending on the altitude and exposure. The seeds mature in August.

Principal variations are in the color of the flowers and in the length of the inflorescence. The petals are usually yellow when fresh, but sometimes very pale, almost white. With age, they tend to become white or pink. The inflorescence is characteristically long and narrow, with ascending slender branches, but this varies with the age of the plant and probably with the habitat.

A plant of *R. T. C. 4952,* the type collection, from Mt. Shasta, is $2n = 30$. No other collections of this subspecies have been investigated cytologically. In shape and size, the chromosomes appear similar to those of ssp. *typicum.*

2. Sedum glanduliferum (Hend.) M. E. Peck, Man. Higher Plants Ore. 361. 1941.

Cotyledon glanduliferum L. F. Henderson, Rhodora **32**: 26. 1930. Type is in the herbarium of the University of Oregon, Eugene, Oregon, from along the trail 3 miles below Alameda, Rogue River, Josephine Co., Oregon, June 1, 1928, *Mr. and Mrs. J. R. Leach 1599.*

Gormania glandulifera L. F. Henderson pro synon., in Peck, ibid.

Perennial with rootstocks to 1 cm. thick, bearing compact clusters of stiff, ascending, rather flat, oblong-spatulate leaves, somewhat glaucous, glandular-crenulate, to 4 cm. long, 1 cm. wide and 2 mm. thick, with apex subacute and grooved; floral stems erect or abruptly decumbent at base, to 3 dm. high, glabrous below, densely glandular above; cauline leaves alternate, oblong-spatulate, sessile, 1.1–2.8 cm. long and 0.5–1.2 cm. wide, glandular-hairy; flowers in a densely glandular two-three parted cyme; floral shoot recurved before anthesis; floral bracts oblong-spatulate, glandular-ciliate, 0.5–1.4 cm. long below, much reduced upwards; pedicels 2 mm. long; sepals erect, ovate or lanceolate, obtuse or acute, united for 1–2 mm. at base, 5–6 mm. long, densely glandular; petals erect, primrose-yellow to greenish yellow, oblong-lanceolate, abruptly acuminate-attentuate, densely glandular,

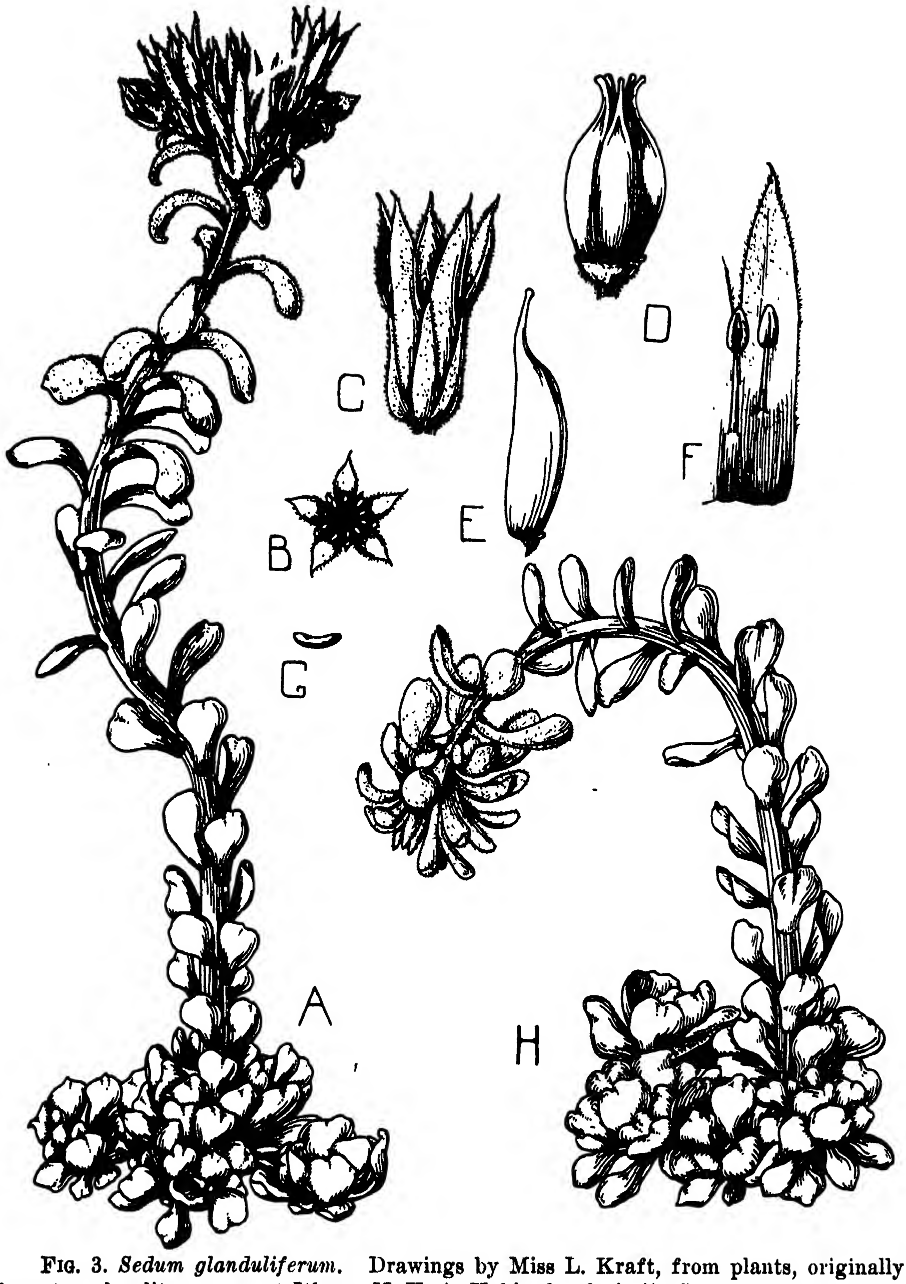

FIG. 3. *Sedum glanduliferum.* Drawings by Miss L. Kraft, from plants, originally from type locality, grown at Ithaca, N. Y. A. Habit sketch ($\times \frac{4}{5}$). B. Flower from above ($\times 1\frac{3}{5}$). C. Flower from side ($\times 1\frac{3}{5}$). D. Carpels ($\times 2\frac{3}{5}$). E. Single carpel ($\times 3\frac{1}{5}$). F. Petal and two stamens ($\times 2\frac{3}{5}$). G. Nectar scale ($\times 4$). H. Habit sketch of plant before flowering ($\times \frac{4}{5}$).

14 mm. long, with the margins connivent for ¼ their length; stamens 4–7 mm. long, with anthers oblong, 0.8–1 mm. long, rounded apically and truncate basally, deep yellow; fresh nectar-scales normally transversely linear; carpels erect, 6–10 mm. long, with short stout styles to 2 mm. long; seeds oblong-pyriform, yellow-brown, finely-striate.—In the original description Henderson commented particularly on the red color of the plant, but I have not been impressed by this character. A plant cultivated in the Santa Barbara Botanic Garden, originally collected by Reid Moran at the type locality, was dark green, only somewhat glaucous. Plants at the type locality, observed by me on July 31, 1940, had the leaves glaucous, somewhat suffused with red or purple, but not decidedly so.

S. glanduliferum is known only from the type locality along the Rogue River below Alameda, Oregon, in the Pacific Border Province (fig. 2).

Specimens seen: Two herbarium collections, three cultures of living plants grown at Ithaca, New York, and one at Santa Barbara, Calif., all from type locality.

Flowering time is June.

Representatives of two collections from the type locality, *Moran 103* and *R. T. C. 5014*, investigated cytologically, are $2n = 30$.

Illustration: Figure 3.

3. Sedum oregonense (Watson) M. E. Peck, Man. Higher Plants Ore. 361. 1941.

Cotyledon oregonensis S. Wats., Proc. Am. Acad. **17**: 373. 1882. The type, in the Gray Herbarium, is the collection of J. and T. J. Howell, no. 322, July 1880, from the Cascade Mts., Oregon. Watson mentioned northern Oregon as the nativity of the species and described the petals as pale yellow. Mt. Hood probably is the original locality. Plants collected by me near Crater Lake closely match the type.

Gormania watsoni Britton, Bull. N. Y. Bot. Gard. **3**: 29. 1903.

Echeveria Watsonii (Britton) Nels. & Macbr., Bot. Gaz. **56**: 476. 1913.

Sedum Watsoni (Britton) Tidestrom, Proc. Biol. Soc. Wash. **40**: 119. 1927.

Perennial with well-developed, much branched, spreading rootstocks to 7 mm. thick, bearing rosettes of fleshy, spatulate leaves, rounded, truncate or slightly retuse at apex, glaucous, yellow-green, often suffused with pink or red, 1–2.8 cm. long, and 0.4–1.1 cm. wide; spreading by the frequent production of offsets of which the stems are green suffused with pink; floral shoots erect, 6–27 cm. high, glabrous, pinkish to red; cauline leaves alternate, oblong-spatulate to almost orbicular, spurred, 0.5–1.2 cm. long; inflorescence paniculate, 1.5–9 cm. long and 1.5–7 cm. wide, usually widest and densest above; floral bracts spatulate, orbicular or linear; sepals ovate, acute, erect, 2–3 mm. long, united for 1 mm. at base; petals oblong, blunt, erect below and connivent for ⅙ to ⅚ their length, somewhat spreading above, 7 to 8 mm. long, white to creamy white; stamens 4–6 mm. long, anthers salmon-pink; nectar scales transversely linear, about 0.8 mm. wide and 0.2 mm. long; carpels 6–7 mm. long, erect.

S. oregonense occurs in the Cascade Mountains of Oregon and northern California, at elevations from 5500 to 7800 ft. (fig. 2). The habitat usually is exposed rocky slopes, cliffs or steep mountain-sides.

Specimens seen: Highest altitude—7800 ± ft., Mt. Jefferson, Ore., *M. E. Peck 9163* (BH); lowest altitude—5200 ft., Mt. Jefferson, Ore., *M. W. Gorman 2831* (DS); northernmost—Bluegrass Ridge, Mt. Hood, Ore., *J. W. Thompson 3323* (DS) (Specimens from bluffs of the ocean, Ilwaco, Pacific Co., Wash., July 26, 1886, *L. F. Henderson 330* (DS) are doubtfully to be referred here. Further material is needed from that locality); easternmost—same as northernmost; westernmost—Preston Peak, Siskiyou Co., Calif., *Doris K. Kildale 8585* (BH, DS); southernmost—Mt. Eddy, Siskiyou Co., Calif., *L. E. Smith 433* (CAS); oldest—1880, Cascade Mts., Ore., *J. & T. J. Howell 322* (GH, NY, DS). Number of collections seen—50.

Flowering time is July; fruiting time, August.

Throughout its range, *S. oregonense* seems constant in its characters. In the horticultural trade, a variety *rosea* is offered.

Two populations from the region of Crater Lake have been investigated cytologically. These both have a $2n$ number of about 90. All other species of the section *Gormania,* which have been studied cytologically, with the exception of one cultivated plant of unknown source and which possibly is this species, have a $2n$ number of 30. Presumably, *S. oregonense* does not interbreed with its relatives, both because of geographic and genetic barriers. No experimental crossing has been attempted. *S. oregonense* is nearest to *S. obtusatum.* The relationships of these two species still require further field study, particularly at places like Mt. Eddy where both occur.

S. oregonense is rather frequent among fanciers as a rock-garden subject. In the trade, it is usually listed as *Gormania watsonii.*

4. SEDUM LAXUM (Britton) Berger.

This is a glabrous perennial with broadly to narrowly spatulate retuse rosette-leaves, pink or pinkish-white flowers and lanceolate, acute or acuminate sepals. The collective species ranges from north-central California to southwestern Oregon and is composed of five subspecies. These are separated in the following key:

A. Plants tall and robust, 25–40 cm. high; leaves dark green, not glaucous; petals deep pink .... 4b. *S. laxum* ssp. *typicum*

AA. Plants not as tall as above, 10–25 cm. high; leaves yellow-green or blue-green, usually glaucous; petals pink, pale pink to white suffused with pink .... B

B. Inflorescence congested; rosettes closely crowded, forming a dense mat, with the leaves rather thin .... 4d. *S. laxum* ssp. *retusum*

BB. Inflorescence lax; rosettes not closely crowded, forming a loose mat, with the leaves thick and leathery C

C. Rosette-leaves very broad, 2.0–3.0 cm. wide, triangular, obcordate, sometimes not glaucous; petals pale pink to white. 4c. *S. laxum* ssp. *latifolium*

CC. Rosette-leaves narrow, 0.3–2.0 cm. wide, oblong-oblanceolate or spatulate, usually glaucous; petals pink .... D

D. Cauline leaves oblong-spatulate, longer than broad. 4a. *S. laxum* ssp. *perplexum*

DD. Cauline leaves cordate to subcordate, about as broad as long .... 4e. *S. laxum* ssp. *heckneri*

4a. SEDUM LAXUM ssp. **perplexum** Clausen, ssp. nov.—Planta parva, 7–

15 cm. alta; folia rosellarum oblonga-oblanceolata vel spatulata, glauca, 0.5–2.5 cm. longa, 0.3–1.4 cm. lata; folia caulina oblonga-spatulata, longiores quam lata; petala roseata.

Flowering plants relatively small, 7–15 cm. high; rosette-leaves oblong-oblanceolate or spatulate, glaucous, rounded, truncate or somewhat retuse at apex, 0.5–2.5 cm. long and 0.3–1.4 cm. wide; rosettes forming loose mats, but not densely crowded; cauline leaves oblong-spatulate, longer than broad; inflorescence a paniculate cyme, broader than long, 2–6 cm. wide and 2–4 cm. long; sepals lanceolate, acute, 4–5 mm. long; petals pink, 8 to 10 mm. long. TYPE in the Bailey Hortorium, from cliff near mouth of Rogue River, Oregon, July 8, 1919, *M. E. Peck 8703*.

Ssp. *perplexum* occurs at low altitudes in the Pacific Border Province from Del Norte Co., California, to Curry Co., Oregon, and inland to Jackson Co., Oregon.

Specimens seen: Highest altitude—6500 ft., Salmon Summit via Horn Creek Trail, Siskiyou Co., Calif., *Doris K. Kildale 5362* (DS); lowest altitude—near sea-level, type collection, *M. E. Peck 8703* (BH, WILLU); northernmost and westernmost—type collection; easternmost—Steen's Butte, Jackson Co., Ore., *M. E. Peck 19429* (WILLU); southernmost—Gordon Mt., Del Norte Co., Calif., *Doris K. Kildale 9930* (BH); oldest—Sept. 11, 1912, trail from Waldo to Black Butte, Siskiyou Co., Calif., *Alice Eastwood 2142* (CAS). Number of collections seen—17. A collection from Trinity Co., Calif., July, 1880 (CAS 136352), would be both the oldest and southernmost record, but the specimens are not sufficiently complete for certain identification.

Flowering time is from June through July. The fruiting shoots and capsules may persist through the winter.

This subspecies passes imperceptibly into the other variants. Specimens intermediate between it and ssp. *typicum* are illustrated by my collection *no. 5015* from a steep slope on the north side of the Rogue River 6 miles above Galice, Josephine Co., Ore. In stature, shape and nature of rosette-leaves and type of inflorescence, this collection stands between the two, but the flowers are white on opening and then become pale pink, in this respect resembling ssp. *latifolium*. Ssp. *perplexum* also shows certain tendencies towards *Sedum oregonense* on the one side and towards ssp. *heckneri* on the other. It may possibly be only a phase of the latter.

Chromosomal data are available for only one collection of ssp. *perplexum*. This is a cultivated specimen of unknown origin. In this, $2n = 30$. A plant of my collection *no. 5015* and a similar intermediate plant from the same region both are $2n = 30$.

From the horticultural trade, ssp. *perplexum* has been received as *Gormania laxa*. It is a desirable subject for the rockery.

4b. SEDUM LAXUM (Britton) Berger, ssp. TYPICUM.

Gormania laxa Britton, Bull. N. Y. Bot. Gard. **3**: 29. 1903. The TYPE, at the New York Botanical Garden, is the collection of Thomas Howell, June 4, 1884, from Waldo, Oregon.

Cotyledon Brittoniana Fedde, Just's Bot. Jahresbericht **31** (1): 827. 1904. Based on *Gormania laxa* Britton.

Echeveria Gormanii Nels. & Macbr., Bot. Gaz. **56**: 476. 1913.

Sedum laxum (Britton) Berger, Engl. & Prantl, Nat. Pflanzenfam. ed. 2, **18a**: 451. 1930.

Stout, with rootstock to 1.5 cm. thick, bearing rosettes of stiff, flat, narrowly spatulate leaves, truncate or retuse at apex, dark green, to 4.5 cm. long and 1.7 cm. wide; floral stems stout, to 1.0 cm. thick, erect throughout development, to 40 cm. high, glabrous; cauline leaves alternate, oblong-spatulate; inflorescence 5–17 cm. long and 10.5 cm. across; floral bracts spatulate to linear; sepals ovate-lanceolate, with their margins connivent below for one-third their length, spreading above, 8 to 10 mm. long; stamens 5 to 6 mm. long; nectar scales transversely linear; carpels erect, with styles somewhat divergent, to 8 mm. long; seeds pyriform, yellow-brown, 1.2–1.5 mm. long.

The typical phase of *Sedum laxum* is known from only two localities, both in Josephine Co., Ore., the region of the type locality, at an elevation of 2000 ft. in the Rogue River Basin, at Waldo, and in yellow pine woods on the east slope of Eight Dollar Mountain, *Elmer Applegate* (DS). I collected and observed the species at Waldo on August 1, 1940 (*R. T. C. 5018*), also I have examined five other collections from that locality. The plant is abundant there on dry rocky slopes. The rosettes are glaucous, green to purple or red, with the leaves finely crenulate and minutely spotted with white. This has the appearance of a distinct species with its large rosette and spatulate cauline leaves, but intermediates occur between the large plants, as they grow at Waldo, and ssp. *perplexum*. Applegate's collection (DS 247453) from near Pilot Rock, Siskiyou Mts., Jackson Co., Ore., illustrates this intergradation.

Two authentic specimens from the type locality, investigated cytologically, are $2n = 30$.

Typical Sedum laxum seems not to be in the trade, although, because of its large size, it is more showy than any of the other subspecies. I have seen one cultivated specimen from a garden in San Francisco. At Waldo, the flowering time is the middle of June.

4c. SEDUM LAXUM ssp. **latifolium** Clausen, ssp. nov.—Planta 15–30 cm. alta; folia rosellarum triangulato-obcordata vel late spatulata, latissima, non glauca vel paulum glauca, 1.5–4 cm. longa, 0.7–3 cm. lata; folia caulina spatulata, longiores quam lata; petala roseata vel alba.

Flowering plants 15–30 cm. high; rosette-leaves very thick, triangular-cordate or broadly spatulate, very broad, deeply emarginate at apex, either yellow-green and not glaucous or somewhat glaucous, 1.5–4 cm. long and 0.7–3 cm. wide; cauline leaves spatulate, longer than wide; inflorescence a paniculate cyme, longer than broad, 2.5–8 cm. long and 1–4 cm. wide; sepals lanceolate, acute, 3–4 mm. long; petals 7–10 mm. long, oblong-lanceolate, pink or white suffused with pale pink. TYPE in the Bailey Hortorium, from rocky slope along Smith River 24 miles northeast of Crescent City, Del Norte Co., Calif., July 24, 1940, *R. T. Clausen 4941*.

Ssp. *latifolium* is at present known only from the general region of the type locality, where it occurs on steep slopes and rocky bluffs at an elevation of about 1500 feet. Besides the collection cited as type, at least seven other collections have been made. The localities for these are Smith River Canyon, Smith River near Adams Station, near Gasquet, Gasquet Mountain, Gasquet

to Patricks and Patrick Creek. Flowering time is late June and early July.

A plant of my collection *4941*, grown in a greenhouse at Ithaca, New York, is $2n = 30$. A plant of a collection by Moran (*no. 406*) from the entrance to the Siskiyou National Forest, 2 miles above Hiouchi Grove on the main highway up the Smith River from Crescent City, Calif., to Grants Pass, Ore., also is $2n =$ *circa* 30.

4d. SEDUM LAXUM ssp. **retusum** (Rose) Clausen, comb. nov.

Gormania retusa Rose. Bull. N. Y. Bot. Gard. **3**: 31. 1903. The TYPE specimen, in the United States National Herbarium, is the collection of A. A. Heller, Aug. 6, 1902, from Sanhedrin Mountain, 5000 ft., Lake Co., Calif.

Gormania Eastwoodiae Britton, Bull. N. Y. Bot. Gard. **3**: 31. 1903. I have seen the TYPE in the herbarium of the California Academy of Sciences and an isotype at the New York Botanical Garden. The type locality is Red Mt., Mendocino Co., Calif.

Cotyledon retusa (Rose) Fedde, Just's Bot. Jahresbericht **31** (1): 827. 1904.

Cotyledon mendocinoana Fedde, ibid. Based on *Gormania Eastwoodiae* Britton.

Sedum sanhedrinum Berger, Engl. & Prantl, Nat. Pflanzenfam., ed. 2, **18a**: 451. 1930.

Sedum Eastwoodiae Berger, loc. cit.

Rootstocks rather stout, bearing many rosettes closely crowded and forming a dense mat; leaves obovate to spatulate, retuse or obtuse, blue-green, glaucous, 2.5 cm. long, or less, thinner than in the other subspecies; floral stems 10–15 cm. tall; inflorescence congested, about 6 cm. long; sepals ovate, acute, 3 mm. long; corolla pink or red, 6–7 mm. long, with the petals connivent for one-fourth to one-third their length.

Range of ssp. *retusum* is in Lake and Mendocino Counties in the Pacific Border Province of northern California. Besides the type and other collections from the original locality, I have seen specimens from Buck Rock Ridge, Mendocino Co., Calif., *Mrs. E. O. Murphey* (CAS).

A plant from Mendocino Co., Calif., no. 28. 3 in University of California gardens, sent by Professor T. H. Goodspeed and cultivated at Ithaca, New York, has a $2n$ number of 30. In the small rosette-leaves and flowers, this seems near to *S. obtusatum,* but in that species the corolla is yellow and the inflorescence is not so dense, also the leaves are not retuse.

Specimens from Klamath, Humboldt Co., Calif. (DS 9854), are intermediate between ssp. *retusum* and *perplexum,* and in their taller stems, tend towards ssp. *typicum.*

4e. SEDUM LAXUM ssp. **heckneri** (Peck) Clausen, comb. nov.

Sedum Heckneri M. E. Peck, Proc. Biol. Soc. Wash. **50**: 121. 1937. TYPE seen in the herbarium of Willamette University, Salem, Oregon. This was collected on a dry cliff along the Middle Fork of the Applegate River four miles above the mouth of Carberry Creek, Jackson County, Oregon, June 26, 1931, *M. E. Peck, no. 16421.*

Plant glaucous; rootstocks stout, freely branched; leaves of the rosettes minutely crenulate, spatulate or obovate—spatulate, truncate to emarginate

at apex, 1–2.5 cm. long; flowering stems 1–2 dm. high, stout, very leafy, with the leaves orbicular to broadly oblong, 0.5–2 cm. long, strongly cordate clasping, spreading at base and curved upward; inflorescence a somewhat globose cluster, 2–4 cm. long and 3–5 cm. wide; calyx 2–4 mm. long, parted nearly to the base, with the lobes ovate, acute or subacute; petals pink, erect, 8–10 mm. long, oblong-lanceolate, obtuse or acute, connivent ¼ their length or sometimes quite free to base; stamens 6–8 mm. long; carpels 8 mm. long; styles divergent.

The distribution is in the Pacific Border Province, in northwestern California and southwestern Oregon, where it occurs on dry cliffs, rocky talus and rocky crests.

Specimens seen: Highest altitude—4590 ft., Horse Mountain, Humboldt Co., Calif., *Doris K. Kildale 2167* (DS); lowest altitude—near sea-level, Rogue River at Gold Beach, Curry Co., Ore., *Doris K. Kildale 6162* in part (DS); northernmost and westernmost—*Kildale 6162;* easternmost—4 miles east of Fork of Salmon, Siskiyou Co., Calif., *Alice Eastwood and J. T. Howell 5056* (CAS); southernmost—*Kildale 2167;* oldest—June 9, 1926, *Kildale 2167*. Number of collections seen—7.

Flowering time is June to July. No information is available regarding the cytology and breeding relationships of this subspecies. It is nearest to ssp. *perplexum,* from which it differs primarily in the shape of the cauline leaves. From ssp. *typicum* it differs in the cauline leaves as well as in the size and form of the inflorescence.

SUMMARY

Gormania is redefined as a subgenus of *Sedum* comprising three sections: *Eugormania, Rosulata* and *Oreganica.* The last is described as a new section. *Eugormania* includes four species. Nomenclatorial innovations are *Sedum obtusatum* ssp. *boreale, S. laxum* ssp. *perplexum, S. l.* ssp. *latifolium, S. l.* ssp. *retusum* and *S. l.* ssp. *heckneri.*

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NOTE:—Since the above was written, Mr. Reid V. Moran has brought to my attention the binomial *Sedum glanduliferum* Gussone, published in 1827. This name is an earlier homonym of *S. glanduliferum* (Henderson) M. E. Peck. Accordingly I take pleasure in renaming as *Sedum moranii* Clausen nom. nov. the species based on *Cotyledon glandulifera* L. F. Henderson, Rhodora **32**: 26. 1930.

69(1): 41–43 JANUARY, 1942

CHROMOSOME STUDIES IN SEDUM, SUBGENUS GORMANIA, SECTION EUGORMANIA

LILLIAN HOLLINGSHEAD

The chromosomes described in this report were studied for Dr. R. T. Clausen to help him in classifying the plants in question, and his conclusions are reported in a paper in this issue of the BULLETIN (Clausen 1942).

Root tips were fixed in chrom-acetic-formalin and imbedded according to Randolph (1935). Sections were cut 8 μ in thickness and stained with crystal violet. The drawings were made with the help of a camera lucida and are reproduced at a magnification of approximately 3500 diameters.

The species and subspecies represented, and the number of plants examined are given in table 1. Chromosome numbers in *S. spathulifolium* are included for comparison, though it belongs to another section of the subgenus (Clausen 1942).

TABLE 1

Chromosome numbers in Sedum, subgenus Gormania

Species	Subspecies	Number of plants	Chromosome number 2n	n
S. obtusatum	*typicum*	2	30	
S. obtusatum	*typicum*	1		15
S. obtusatum	*boreale*	1	30	
S. glanduliferum		2	30	
S. oregonense		3	90 ±	
S. laxum	*perplexum*	1	30	
S. laxum	*typicum*	2	30	
*S. laxum**		1	30	
*S. laxum**		1	30	
S. laxum	*latifolium*	2	30	
S. laxum	*retusum*	1	30	
S. spathulifolium		3	30	

* Intermediates between *perplexum* and *typicum*.

The diploid number is 30 throughout excepting *S. oregonense* in which the three plants examined have about 90 chromosomes. These plants are, therefore, hexaploid in relation to the other species.

The chromosomes in all species are very small, and in late prophase and at metaphase appear roughly circular or elliptical in cross section (figs. 1–5). There is no consistent visible evidence of the two-armed structure, typical of mitotic chromosomes in general, and which has been found char-

acteristic of *Sedum,* even in those species which have the smallest chromosomes (Baldwin 1935, 1936, 1937, 1939, 1940). Moreover one plant of *S. debile* which Britton included in *Gormania* (Clausen 1942) has much larger markedly two-armed chromosomes quite different from those of *Gormania.* Dr. Clausen includes it with *S. divergens* which preliminary studies show has about 16 diploid two-armed chromosomes.

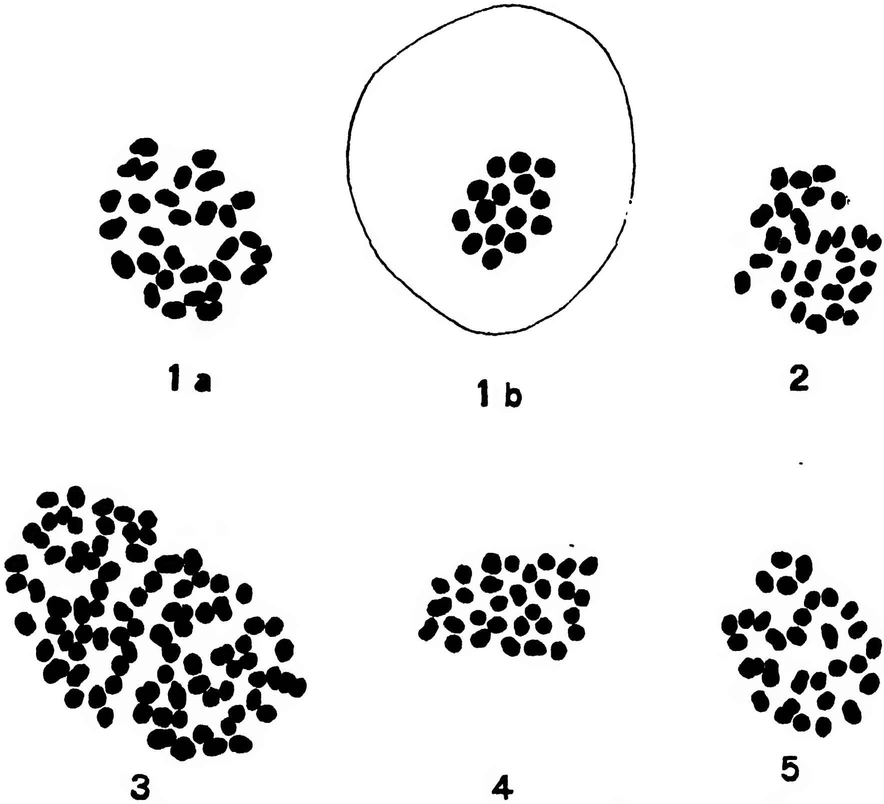

FIG. 1. *S. obtusatum typicum.* (a). Mitotic metaphase ($2n=30$). (b). Meiotic metaphase ($n=15$). FIG. 2. *S. glanduliferum* ($2n=30$). FIG. 3. *S. oregonense* ($2n=90\pm$). FIG. 4. *S. laxum typicum* ($2n=30$). FIG. 5. *S. spathulifolium* ($2n=30$).

As metaphase proceeds, each chromosome divides into two daughter chromosomes roughly spherical in shape and they move apart to the poles, in appearance very much like tiny meiotic chromosomes. The constricted appearance of some chromosomes in the figures represents in each case the polar view of a dividing chromosome in which one daughter lies slightly to one side of the other.

The outlines are not so clear as the black and white figures indicate, but in each plant recorded metaphases were found in which the chromosomes

were well separated and readily countable, except in the case of those with 90 odd chromosomes which are not quite so clear.

There are apparent size differences among the chromosomes in one plant, which may be due in part to differences in orientation. No particular chromosomes could be identified. Neither could the complexes of the 30-chromosome species be distinguished from one another. Metaphases can be found in which the chromosomes appear to be larger than others of the same species. This can arise from the fact that early metaphase chromosomes appear larger than they do at late metaphase. As metaphase proceeds and each chromosome prepares to divide, it becomes oriented with the axis of division strictly toward the poles and the upper small daughter chromosome wholly obscures the one below, whereas earlier the orientation may be such as to show parts of both daughter chromosomes.

The one plant (*S. obtusatum*) examined at meiosis had the expected 15 pairs of chromosomes (fig. 1 b).

Baldwin (1939), who has made numerous studies in Crassulaceae, has found this family very diverse karyologically, but nevertheless within restricted groups, chromosome size is fairly uniform. The study here reported supports his conclusions, for the chromosomes throughout the group have a characteristic form and size. It further illustrates the value of a cytotaxonomic approach to an understanding of the family.

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BULLETIN OF THE TORREY BOTANICAL CLUB
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NEW AND UNUSUAL CORTINARII FROM MICHIGAN, WITH A KEY TO THE NORTH AMERICAN SPECIES OF SUBGENUS BULBOPODIUM[1]

ALEXANDER H. SMITH

During the last part of August and the first week in September of 1940, southeastern Michigan received an exceptionally heavy rainfall. As a result, agarics fruited prolifically during September even though the weather during the remainder of the month was no wetter than usual. The most conspicuous element in the agaric flora was the numerous species of the genus *Cortinarius*. On no previous occasion, either in the Olympic Mountains of Washington or along the northern coast of California where I (7) had found *Cortinarii* most abundantly, have I seen so many species or such large quantities of material as appeared around Ann Arbor early in the fall of 1940. There were relatively few members of the subgenera *Myxacium, Inoloma,* and *Dermocybe,* but this lack was more than offset by *Bulbopodium, Phlegmacium, Telamonia,* and *Hydrocybe.* Nearly all the *Cortinarii* fruited within the space of two weeks, so that it was possible to study only a limited number. In the following account particular emphasis has been given to *Bulbopodium,* a subgenus to which I have paid special attention for the past seven years. In the other subgenera only those species with some outstanding macroscopic character have been included. By limiting myself to such species, the descriptions of the earlier mycologists, who did not study microscopic characters, can be used to some advantage.

DISCUSSION OF AND KEY TO THE NORTH AMERICAN SPECIES OF BULBOPODIUM

Since particular attention was paid to the species of *Bulbopodium,* I have given, in the following account, a list of all the members of that subgenus which I collected during the fall of 1940, a list of the additional species which at one time or another I have seen in the fresh condition, and also a list of those recognized from North America by Kauffman (3), but of which I have not seen fresh material. As a summary, a revised key to the known North American species is included. Because of lack of space, descriptions are given only for the new species or in cases of revised concepts. Descriptive accounts of the others are to be found in Kauffman (3) and in Smith (7). Murrill's (6) new species of *Cortinarius* are not included. Of those apparently belonging to *Bulbopodium, C. subfulmineus* keys out to *C. fulgens* and may possibly be that species. *C. subglaucopus* is undeterminable because he has not given the color of the young gills.

[1] Papers from the University of Michigan Herbarium.

The following is the list of species collected in the vicinity of Ann Arbor during the fall of 1940: *C. aggregatus, C. albidus, C. amnicola, C. arenicola, C. Atkinsonianus, C. Bouderi, C. caesiocyaneus, C. calyptrodermus, C. citrinipedes, C. corrugatus, C. elegantioides, C. fulmineus* var. *sulphureus, C. michiganensis, C. purpureophyllus, C. olivaceostramineus, C. squalidus, C. subsolitarius,* and *C. virentophyllus.*

In addition to the eighteen listed above, I have previously seen material of *C. arquatus, C. calochrous, C. calyptratus, C. cedretorum, C. glaucopus, C. herpeticus, C. intrusus, C. metarius, C. montanus, C. multiformis, C. occidentalis, C. olympianus, C. orichalceus, C. Parksianus, C. prasinus, C. purpurascens, C. scaurus, C. subpurpurascens, C. subpurpureophyllus, C. velicopia, C. volvatus.*

Of those included by Kauffman (3) in his monograph, I have not seen fresh specimens of *C. caerulescens, C. sphaerosperma, C. lilacinopes, C. glaucopoides, C. citrinellus, C. fulgens, C. elegantior, C. rubens,* and *C. sublateritius. C. fulgens* and *C. elegantior* are included in this last group simply because my collections have not checked well with existing descriptions, and I have no clear species-concepts of them based upon fresh material.

Of the thirty-four *Bulbopodia* recognized for North America by Kauffman, I have seen twenty-five in the fresh condition. Fourteen additional species have been added bringing the total number recognized for the continent up to forty-eight. In the descriptive text, all color terms within quotation marks are taken from Ridgway, *Color Standards and Color Nomenclature,* Washington, D. C., 1912. Collections cited by number have been desposited in the University of Michigan Herbarium.

Revised Key to the North American Species of Bulbopodium

1. Lamellae (and at least the apex of the stipe) at first violet purplish, blue or shades of these colors. (*C. Atkinsonianus* and *C. cedretorum* with yellowish gills are included here, as is *C. arenicola* with pallid gills) .... 2.
1. Lamellae at first green, olivaceous, smoky or assuming shades of these colors (context and young lamellae may be slightly violaceous or blue at first in *C. montanus, C. scaurus* and *C. herpeticus*) .... 30.
1. Lamellae yellow, fulvous, cinnamon reddish or dull rusty brown when young .... 35.
1. Lamellae white or pallid at first .... 42.

Lamellae at first violaceous, lilac or purplish

2. Lamellae turning purplish when bruised .... 3.
2. Lamellae not turning purplish when bruised .... 6.
3. Stipe stuffed to hollow; context not turning purplish; pileus yellow-ochraceous tawny .... *C. subpurpurascens.*
3. Stipe solid, context turning purplish when bruised .... 4.
4. Spores spheroid, 7–8 × 6–7.5 μ; pileus violet-purple .... *C. sphoerosperma.*
4. Spores ellipsoid .... 5.
5. Pileus blue when young, fading to gray in age .... *C. occidentalis.*

5. Pileus umber-purplish, soon streaked with clay-color or dark reddish brown. *C. purpurascens.*
6. Spores averaging more than 10 μ long .... 7.
6. Spores not more than 10 μ or rarely 11 μ long .... 20.
7. Surface of pileus conspicuously corrugated .... *C. corrugatus.*
7. Surface of pileus smooth or slightly uneven .... 8.
8. Pileus chiefly violet or purplish .... 9.
8. Pileus if violet or purplish quickly changing to some other color .... 12.
9. Universal veil ochraceous buff, stipe and pileus lutescent .... *C. velicopia.*
9. Universal veil if present white; pileus and stipe violaceous, hardly changing .... 10.
10. Young gills faintly tinged pinkish, pileus and stipe pinkish lilac overall ... *C. arenicola.*
10. Young gills dark or pallid violet .... 11.
11. Gills pallid to pale violet, pileus glabrous or slightly fibrillose .... *C. caesiocyaneus.*
11. Gills dark violet, pileus decorated with white felt-like masses of universal veil tissue. *C. calyptrodermus.*
12. Pileus normally 4–6 (7) cm. broad .... 13.
12. Pileus normally 6–12 cm. broad .... 17.
13. Lamellae broad, violet amethyst; pileus soon yellow, bulb white, spores 12–14 × 6–7 μ. *C. caerulescens.*
13. Not as above .... 14.
14. Stipe 8–15 cm. long, 10–15 mm. thick, pileus dark reddish brown. *C. subpurpureophyllus.*
14. Stipe shorter, pileus yellow .... 15.
15. Universal veil brilliant orange, stipe pinkish lilac above, bright yellow below both inside and out .... *C. amnicola.*
15. Universal veil at most merely pale yellowish .... 16.
16. Spores 9–12 (13) × 5–6 μ .... *C. metarius.*
16. Spores 11–14 (15) × 6–8 μ .... *C. arquatus.*
17. Spores averaging more than 12 μ long, surface of pileus yellow .... 18.
17. Spores 10–12 (13) μ long, pileus variously colored .... 19.
18. Context pale incarnate, stipe white downwards, without evident universal veil. *C. lilacinopes.*
18. Context deep violet, pileus and universal veil bright olive yellow. .... *C. Atkinsonianus.*
19. Pileus dull purplish umber when young .... *C. Parksianus.*
19. Pileus tawny-reddish when young, gills bright lavender .... *C. purpureophyllus.*
19. Pileus olive-buff when young, somewhat testaceous in age, gills rather dark grayish lavender .... *C. Boudieri.*
19. Pileus bright yellow, becoming dark red at maturity, lamellae yellow at first. *C. cedretorum.*
20. Pileus 3–7 cm. broad (occasionally larger) .... 21.
20. Pileus 6–12 cm. or more broad .... 27.
21. Pileus covered by a white felt-like mass of universal veil tissue at least over the disc, flesh pallid brownish, surface violet .... *C. calyptratus.*
21. Pileus glabrous or fibrillose streaked, if with universal veil remnants the latter not becoming white .... 22.
22. Disc of pileus bright yellow or streaked with greenish yellow due to greenish yellow veil remnants .... 23.
22. Pileus differently colored, usually violet to whitish .... 24.
23. Pileus glabrous, bright yellow .... *C. calochrous.*
23. Pileus olivaceous yellow to sordid yellow over the disc, dark purplish brown toward the margin .... *C. citrinipedes.*
24. Usually densely gregarious or cespitose .... 26.
24. Usually solitary or scattered, occasionally 2 or 3 in a cluster .... 25.
25. Universal veil greenish yellow .... *C. citrinipedes.*

25. Universal veil lacking, cortina dark violet, pileus gills and stipe dark violet and hardly fading .... *C. subsolitarius.*
25. Universal veil copious and white, pileus becoming whitish .... *C. volvatus.*
25. Gills pinkish lilac, universal veil inconspicuous .... *C. olympianus.*
26. Gills pinkish lilac, retaining a lilac tinge .... *C. olympianus.*
26. Gills violet-purple at first, soon pale purplish gray .... *C. aggregatus.*
27. Bulb large and prominently depressed, plant pale violaceous .... *C. michiganensis.*
27. Bulb relatively small, lamellae rather broad .... 28.
28. Context, pileus and stipe at first violet-purple, pileus at length smoky olive-gray. *C. aggregatus.*
28. Context whitish, at length lutescent .... 29.
29. Pileus pale-orange-yellow, not streaked .... *C. glaucopoides.*
29. Pileus slate gray, fulvous-streaked, often greenish when young or greenish gray. *C. glaucopus.*

Lamellae at first green or olivaceous or soon becoming so

30. Context and young lamellae at the very first tinged with violet, blue or purplish but lamellae very soon olivaceous .... 31.
30. Context and gills not as above .... 33.
31. Spores 9–11 (12) × 5.5–6.5 μ, pileus variegated with brown, tawny and slight olivaceous hues .... *C. montanus.*
31. Spores 8–9.5 × 5–6 μ; pileus Dresden-brown to tawny .... 32.
32. Bulb yellow from universal veil; stipe 6–10 mm. thick, lamellae narrow .... *C. scaurus.*
32. Bulb white or whitish; stipe 10–20 mm. thick, lamellae broader than the preceding. *C. herpeticus.*
33. Stipe bluish, pileus and gills green .... *C. virentophyllus.*
33. Stipe not bluish .... 34.
34. Disc of cap spotted dark vinaceous red or becoming reddish over all .... *C. orichalceus.*
34. Pileus remaining dark olive-color in age or when dried .... *C. prasinus.*

Lamellae at first yellow, fulvous, cinnamon reddish or dull brown
(But see *C. Atkinsonianus* & *C. cedretorum* under 2 in this key.)

35. Growing in greenhouses .... *C. intrusus.*
35. Growing in the woods .... 36.
36. Spores not more than 10 μ long .... 37.
36. Spores averaging more than 10 μ long .... 38.
37. Spores ellipsoid, pileus and stipe sulphur yellow; arising from a yellow mycelium. *C. fulmineus* var. *sulphureus.*
37. Spores subglobose, 8–9 × 6–8 μ, pileus at first buff-citrine, then clay-colored. *C. citrinellus.*
37. Spores ellipsoid, gills dull brown, pileus dark brown in age .... *C. squallidus.*
38. Taste of context decidedly bitter; pileus and universal veil yellow; spores 15–18 (20) × 7–9 μ .... *C. elegantoides.*
38. Taste not bitter .... 39.
39. Pileus corrugated, tawny to yellowish .... *C. corrugatus.*
39. Pileus not corrugated .... 40.
40. Spores more than 12 μ long .... 41.
40. Spores 9–12 μ; pileus bright orange (*C. cedretorum* may key out here also, cap yellow, flesh lilac) .... *C. fulgens.*
41. Pileus yellow, spores 12–15 μ long .... *C. elegantior.*
41. Pileus dull red to orange-tawny; spores 15–18 × 7–8.5 μ .... *C. rubens.*

Lamellae pallid at first

42. Pileus pale olivaceo-stramineus, stipe soft, becoming hollow .... *C. olivaceostramineus.*

42. Pileus without olive tints .... 43.
43. Spores not more than 9 μ long; pileus white-hoary, lutescent to rusty orange. *C. multiformis.*
43. Spores more than 9 μ long .... 44.
44. Pileus lilac, becoming brownish, gills pallid or with a slight pinkish tinge. *C. arenicola.*
44. Pileus and stipe white .... *C. albidus.*
44. Pileus yellowish, brownish or red at first .... 45.
45. Pileus light red, pellicle not bitter .... *C. sublateritius.*
45. Pileus yellowish, pellicle bitter .... *C. aleruiosmus.*[2]

NEW OR REDESCRIBED BULBOPODIA

Cortinarius amnicola Smith, sp. nov. Pileus 4.5–7 cm. latus, convexus demum planus, viscidus, laete luteus et aurantio-virgatus; lamellae lilacinae, confertae, angustae; stipes 4–7 cm. longus, 8–12 mm. crassus, marginato-bulbosus, solidus, deorsum laete luteus, sursum lilacinus; sporae 10–12 × 6–7 μ.

Pileus 4.5–7 cm. broad, broadly convex to subhemispheric when young, becoming plane, viscid, appearing as if streaked with agglutinated fibrils, color evenly "capucine yellow" or with "Mikado orange" streaks (very brilliant yellow, often streaked with orange-yellow), veil remnants sparse and merely scattered along the margin; flesh pallid in the pileus, "pale vinaceous lilac" in the upper part of the stipe, odor faintly fragrant, taste mild in all parts; lamellae "pale vinaceous lilac" (pale lilac) at first, slowly changing to "vinaceous fawn" (pale lilac-brown), sometimes "vinaceous lavender" (dull lilac) in partly expanded pilei, moderately close, deeply adnexed, narrow (5 mm. ±), moderately close, edges even; stipe 4–7 cm. long, 8–12 mm. thick, solid but becoming spongy in the bulb in age, bulb up to 2 cm. broad and depressed-marginate, surface "pale vinaceous lilac" toward the apex, bright yellow both inside and out toward the base, silky from the veil remnants, fragments of universal veil adhering to the margin of the bulb and nearly "Mikado orange" in color, base of bulb and mycelium "citron-yellow," cortina scanty and very pale yellowish; spores 10–12 × 6–7 μ, tuberculate-roughened, pale russet in KOH, narrowly amygdaloid; basidia four-spored; cheilocystidia and pleurocystidia not differentiated; pileus-trama with a thick gelatinous pellicle made up of narrow (2–4 μ) contorted hyphae with dull lilac contents when mounted in KOH, contents rusty brown in chloral hydrate and iodine.

Scattered on humus under butternut, walnut, and basswood on low ground along streams, Ann Arbor, Sept. 11 (*A. H. Smith 15308*), and Sept. 15, 1940 (*A. H. Smith 15381*—TYPE).

The pilei of this species assume a dark vinaceous red color ("ocher-red") when dried in contrast to the bright or pale yellow of dried pilei of *C. calochrous*. The brilliant colors of the stipe, if preserved by careful drying, fade in short time, and in herbarium specimens soon become a dull grayish-olivaceous hue. *C. amnicola* is distinct from *C. calochrous* as the latter is known

[2] *Cortinarius aleuriosmus* sensu Kauffman is here keyed out as *C. Boudieri*; the true *C. aleuriosmus* is not yet known from North America.

in the United States, by the color change which takes place as specimens are dried, by its larger spores and persistent lilac color in the apex of the stipe. My concept of *C. calochrous* is based on Kauffman's (3) account and on material which corresponds to it which I have collected both in Michigan and along the west coast. Lange's (5) concept and that of Kauffman agree closely and certainly apply to the same species. Henry (1) on the other hand has described a species practically identical with *C. amnicola* under the name *C. calochrous.* I have no information on the colors assumed in drying by the pilei of his specimens, so no comparison can be made on that point. Since Fries and most European workers after him have not placed a fungus with persistent lilac colors in the apex of the stipe in *C. calochrous,* I have described my collections as a different species. A sharp difference in spore-size has been observed between the local collections of *C. amnicola* and *C. calochrous.* This difference is not so apparent when one examines the descriptions of the latter, however. The spores of *C. calochrous* are generally given as 10–11 μ long by European authors. Kauffman (3) stated that they seldom measured 10 μ long. In my western specimens they measure 8–10.5 μ, are very pale brown in KOH and only slightly roughened. However, when compared under the microscope, the spores of *C. amnicola* are larger, darker, more tuberculate and more amygdaloid in shape. These differences appear to me to be taxonomically significant.

Cortinarius arenicola Smith, sp. nov. Pileus 3–5 cm. latus, convexus demum subplanus, viscidus, lilaceus demum sordide lilaceus vel brunneo-maculatus; caro albida; lamellae pallidae demum sordide incarnato-brunneae; stipes 3–5 cm. longus, 8–10 mm. crassus, marginato-bulbosus, lilaceus; sporae 9–11 (12) × 5.5–6.5 μ.

Pileus 3–5 cm. broad, convex, becoming plane or the disc flattened and the sides arched, viscid, "vinaceous lilac" over all at first, fading slowly to "pale vinaceous lilac" or pallid in spots where covered by leaves, margin inrolled and delicately white fibrillose from the pallid cortina, sometimes with small spot-like scales over the disc, old pilei occasionally developing sordid brownish spots; flesh white or faintly lilac just beneath the cuticle, no color change when bruised, odor none, taste mild; lamellae close, moderately broad (5 mm. ±), sharply and deeply adnexed, 2–3 tiers of short individuals, color in button stages "pale vinaceous fawn" (pallid), soon sordid brownish, at maturity near "fawn-color," edges whitish and serrulate to eroded; stipe 3–5 cm. long, 8–10 mm. thick, equal above a small depressed-marginate bulb, solid, flesh lilac near the periphery, whitish in the center, surface evenly "vinaceous lilac" over all, base of bulb paler lilac to whitish, cortina white and causing the surface of the stipe to appear whitish in young stages; spores 9–11 (12) × 5.5–6.5 μ, amygdaloid, dark rusty brown under the microscope in KOH, tuberculate; basidia four-spored; cheilocystidia and pleurocystidia not differentiated; pileus with a gelatinous pellicle, the hyphae 4–5 μ thick and yellowish cinnamon in KOH.

Singly under sassafras, in a dry sandy open woods, Waterloo Project,

Waterloo, Sept. 12, 1940 (*A. H. Smith 15315*—TYPE). Known only from the one locality.

The outstanding characters of *C. arenicola* are the rosy-lilac colors of pileus and stipe, the pallid gills, the very small but depressed-marginate bulb, and spores. *C. olympianus* can be readily distinguished when fresh by the pale bright lilac color of the young gills and clearer color of the pileus. Its pilei dry whitish instead of lilac-brown as in *C. arenicola*. The difference in habitat, of course, is striking but in my estimation is not taxonomically significant.

C. arenicola is distinct from *C. caerulescens* in its pallid gills which become vinaceous brown ("fawn-color") as they mature. The colors of the pileus and stipe also differ from those generally attributed to *C. caerulescens*. *C. olympianus* and *C. caerulescens* have the same appearance when dried but differ sharply in spore size. Specimens of *C. arenicola* dry differently from either of these two and have spores intermediate in size. *C. Dionysae* has the same stature as *C. arenicola* and the same sized spores but differs in the color of the gills and in having a distinctive odor. *C. sodagnitus* has larger spores and lilac gills which are apparently similar in color to those of *C. calochrous*. *C. nemorosus* sensu Henry has larger spores, 13–14 × 6.5–7 μ, and *C. dibaphus* sensu Henry has lilac gills and larger spores.

CORTINARIUS BOUDIERI Henry. Pileus 5–13 cm. broad, broadly convex to nearly plane when young, the margin strongly inrolled, sometimes becoming plane, sometimes with a low obtuse umbo, occasionally the margin becoming wavy but frequently remaining decurved and inrolled, surface viscid to glutinous, the margin often streaked with the fibrillose remains of the veil, the disc often developing spot-like scales, otherwise glabrous, evenly colored, near "pale olive-buff" or pale grayish buff over all when young (colors not matching well in Ridgway), with a tinge of lilac pervading, soon developing vinaceous brown colors either on the disc or along the margin or over all, color then more or less "vinaceous buff" or "avellaneous" but tending toward "fawn-color" (colors very sordid pale vinaceous tawny to avellaneous, no good match in R.), spot-like scales sometimes darker; flesh thick and firm, "pale brownish drab" (sordid brownish lilac), sometimes faintly yellowish near the apex of the stipe, becoming paler but retaining a lilac-brown tinge, odor none, taste mild, no color change when cut or bruised (sordid darker brown or yellowish around the wormholes); lamellae "pale vinaceous drab" to "light vinaceous drab" (dull purplish brown), changing very slowly to sordid pale cinnamon-brown or nearly "army-brown," not staining when bruised, close, 2–3 tiers of short individuals, rather narrow, (8 mm. in a 12 cm. cap), sharply adnexed, edges a bit eroded at times; stipe short, 2–4 (7) cm. long, (10) 15–30 mm. thick above the broad (3–5 cm.), flat, marginate but not depressed bulb, solid, flesh very firm and sordid watery lilac (very pale), soon lilac-buff or whitish (similar to that of the pileus), surface very silky and pale lilac, cortina whitish and not copious, bulb whitish and flattened on the underside, universal veil scanty, when well developed adher-

ing along the margin of the bulb in inconspicuous patches, the margin of the bulb sometimes sordid yellowish as if stained; spores 9–11 × 5–6 μ, very rarely 12 × 7 μ; subamygdaloid, dark rusty brown under the microscope, rather coarsely tuberculate; basidia four-spored, hyaline or slightly yellowish in KOH; cheilocystidia and pleurocystidia not differentiated; pileus-trama with a gelatinous pellicle of hyaline (in KOH) contorted hyphae 4–6 μ thick.

Cespitose to gregarious in arcs under oak, hickory, basswood and sassafras, Ann Arbor, Sept. 13–17, 1940 (*A. H. Smith 15328, 15330, 15393, 15332, 15382*).

This species has the same stature as *C. purpureophyllus* and also approaches the latter in color. The colors are consistently much paler and duller, however, and there is a slight difference in spore-size as well. Dried specimens of the two species can be very easily distinguished in the herbarium by the reaction of the hymenium to KOH. In *C. purpureophyllus* the basidia become bright pink, near "Rose Doree" whereas in *C. Boudieri* they remain hyaline or pale yellowish. Both species were found in the one locality and compared when fresh as well as after being dried. The reaction of the hymenium in KOH has, of course, been verified by a study of the type of *C. purpureophyllus*.

The white flesh, farinaceous odor and bitter taste of the pellicle of the pileus distinguish *C. aleuriosmus* Maire. In addition, the colors of the pileus in *C. Boudieri* become much darker than those of *C. aleuriosmus* as described by Maire. Maire has given the name *C. Rickenianus* to the species Ricken placed under *C. aleuriosmus*. It appears to me, on the basis of the descriptions, that Ricken's concept of *C. aleuriosmus* and Boudier's of *C. multiformis* represent the same species for which the earliest name is *C. Boudieri*.

Cortinarius calyptrodermus Smith, sp. nov. Figure 1. Pileus 5–10 cm. latus, convexus demum planus, viscidus, fragmentis volvae obtectus, laete caeruleus, demum pallide caeruleus; caro pallide lilacina vel subcaerulea; lamellae intense caeruleae; stipes 5–9 cm. longus, 1–2.5 cm. crassus, marginato-bulbosus, pallide caeruleus demum albido-caeruleus; sporae 10–12 × 6–7 μ.

Pileus 5–10 cm. broad, convex to obtuse in button stages, broadly convex to plane in age, the margin streaked with agglutinated fibrils, whitish to pale lilac in button stages (the universal veil almost completely covering the young cap), the universal veil tissue becoming areolate-cracked and finally aggregated into matted fibrillose patches at least around and over the disc, the disc sometimes covered with a large felt-like calyptra, viscid beneath the remains of the veil or when the latter have been worn away, color "pale bluish lavender" over all in button stages (owing to the colored u. veil), pileus "Vanderpool's violet" (deep violet) beneath the veil, becoming "light full bluish violet" over all at maturity, in age slowly becoming whitish or sordid brownish; flesh pale lilac (like the u. veil) or brighter in the apex of the stipe and there colored like the surface of the pileus, finally becoming very pale lilac or whitish in both pileus and stipe, unchanging when bruised,

moderately thick and firm, odor and taste not distinctive; lamellae moderately close and broadly adnexed, "Vanderpool's violet" when young, retaining this color very persistently and in age only tinged with brown, the edges decidedly eroded; stipe 5–9 cm. long, 1–2.5 cm. thick above the broad marginate bulb, bulb 3–4 cm. broad, pale violet at first from the universal veil remnants, whitish in age, rounded below, violaceous within and later becoming white, concolorous with the gills above the bulb and beautifully decorated with the remnants of the violet cortina, solid, firm; spores $10–12 \times 6–7\ \mu$, subamygdaloid, dark tawny under the microscope in KOH, coarsely tuberculate; basidia four-spored; cheilocystidia and pleurocystidia not differentiated or the former merely slightly larger than the basidia but of the same shape; pileus-trama homogeneous beneath a thin gelatinous pellicle, composed of hyphae $4–7\ \mu$ broad which have hyaline to pale vinaceous contents in KOH; at times with a rather strong vinaceous tint throughout the entire thickness of the pellicle.

Gregarious in arcs in a low woods of second growth oak and basswood and various shrubs, Sharron Hollow, Sept. 14, 1940 (*A. H. Smith 15356*—TYPE). Known only from the one locality, but a wagon load of material could have been collected that day. It was the most abundant mushroom in the woods.

One outstanding feature of this species is the mass of white mycelium which surrounds the base of the bulb. This mycelium penetrates through the humus and debris causing much of the latter to be held together in a manner similar to that of *Tricholoma acerbum.* The very well-developed universal veil is, of course, the most conspicuous feature of the fungus. Hundreds of arcs, each containing anywhere from ten to fifty fruiting bodies were observed on Sept. 14, and the veil was a very constant feature. The only other species in which I have observed such a character is *C. calyptratus* from California. The latter is distinguished both by its brownish flesh and smaller spores. *C. praestans* is also said to have a well developed veil which leaves patches of tissue over the pileus, but it does not belong in the subgenus Bulbopodium. I regard the extreme development of such a tissue in viscid Cortinarii as a secondary character lacking any particular phylogenetic significance but furnishing a valuable aid in recognizing the species.

Henry (1) gives the spores of *C. caerulescens* as $10–13 \times 6–7\ \mu$, and describes the bulb and surface of the pileus as becoming yellowish in age. I did not observe such color changes in my collection or in any of the material seen in the field at the time. It is very likely that this color change is a good character by which the two can be separated, but I have hesitated to emphasize it. The fruiting bodies of the various species collected along with *C. calyptrodermus* had developed during about ten days of clear weather following a period of very heavy rainfall. In all, the bright colors characteristic of the young stages were unusually persistent in the maturing pilei. Since the change to yellow in *C. caerulescens* apparently develops as the specimens mature and age, this change may have been inhibited by the conditions pre-

FIG. 1 *Cortinarius calyptrodermus* Smith. × 1.

vailing last September, and *C. calyptrodermus* may show it on subsequent collections. *C. Atkinsonianus* was very abundant in the same locality and showed scarcely any change to reddish on the pilei of old specimens. Ordinarily this change is very conspicuous. However, in drying, the specimens of the latter species did turn reddish. Those of *C. calyptrodermus* dried a dull blue and now appear very different from dried material of *C. caerulescens.*

C. michiganensis is similar to *C. calyptrodermus* in stature but has smaller spores and generally a shorter stipe. *C. caesiocyaneus* has much paler gills when young and a glabrous pileus in age.

Cortinarius citrinipedes Smith, sp. nov. Figure 3. Pileus 4–7 cm. latus, late convexus, demun planus, viscidus, sordide olivaceo- vel luteo-fibrillosus, glabrescens, obscure purpureo-brunneus demum subargillaceus; caro obscure lilacina; lamellae obscure lilacinae demum argillaceae; stipes 4–6 cm. longus, 1–2 cm. crassus, marginato-bulbosus, solidus, obscure lilacinus, argente fibrillosus; sporae 8–10 (11) × 4.5–5 μ; mycelium olivaceo-luteum.

Pileus 4–7 cm. broad, broadly convex with an inrolled margin, remaining broadly convex or expanding to nearly plane, the margin remaining inrolled for a long time, surface viscid, soon dry, buttons "Isabella color" (sordid greenish yellow) on the disc from the universal veil remnants, "benzo-brown" (dark purplish brown) near the margin, sometimes with a pale pruina over all causing it to appear as though with a lilac-olivaceous sheen, in age becoming clay color on the disc or with a persistent greenish yellow cast, sometimes unicolorous and dark purplish brown (when the veil remnants have disappeared), inrolled margin more or less lilac-gray; flesh moderately thick, "dark lavender" (a dull fairly deep lavender) and slowly becoming whitish, not staining where bruised, odor and taste not distinctive; lamellae "dark lavender" when young and slowly changing to "tawny-olive" (dull yellowish brown, never truly yellow), bluntly adnate and very narrow (4 mm. broad ±), crowded, edges even; stipe 4–6 cm. long, 1–2 cm. thick, with a marginate bulb 3–4 cm. broad, solid, flesh "dark lavender" but becoming whitish, surface pale silvery lilac from the copious lilac-white cortina, universal veil and mycelium around the base of the bulb "greenish yellow" (almost more greenish than yellow) and causing the base of the bulb to be bright greenish yellow in contrast to the other parts; spores 8–10 (11) × 4.5–5 μ, about ochraceous tawny under the microscope in KOH, narrowly amygdaloid, slightly roughened; basidia four-spored; cheilocystidia not differentiated; pileus-trama with a thin gelatinous pellicle made up of contorted hyphae 3–5 μ thick.

Gregarious on humus in oak woods, Ann Arbor, Sept. 11, (*A. H. Smith 15305*—TYPE); Sharron Hollow, Sept. 14 (*15358*), and Ann Arbor, Sept. 16, 1940 (*15395*).

The sordid greenish yellow color on the disc of the pileus is caused by adhering remains of the greenish yellow universal veil. These do not form a tissue as in *C. calyptrodermus,* but rather become appressed and somewhat dispersed giving the disc of the pileus an appressed-fibrillose appearance and obscuring the true color. Beneath the veil-remnants the pileus is evenly

colored. When dried the pilei vary from olivaceous gray to lilac gray on the margin and dark rusty brown over the disc. In none were the colors as bright or as yellowish as in *C. glaucopus*. The dark, dull lavender flesh and also the lavender gills are very characteristic. The bright greenish yellow universal veil and mycelium is in decided contrast to the color of all other parts. These characters in addition to the small spores distinguish the species.

C. velicopia Kauff. is very close to *C. citrinipedes* but is characterized by cheilocystidia, paler bluish colors instead of lavender, and a tendency for the pileus to become yellowish. There is also a slight but appreciable difference in spore size when spores of both are compared under the microscope, and a distinct difference in the color of their universal veils.

Cortinarius squalidus Smith, sp. nov. Figure 4. Pileus 3–6 (8) cm. latus, convexus, demum planus, viscidus, subcanus demum glaber, pallide cinnamomeus demum obscure brunneus; lamellae pallide brunneae nunquam albidae, demum obscure brunneae; stipes 3–5 cm. longus, 10–15 mm. crassus, solidus, bulbosus, sericeo-fibrillosus et pallidus; sporae 7–9 × 4–5 μ.

Pileus 3–6 (8) cm. broad, convex, obtuse in age, the margin incurved or bent in slightly and usually remaining decurved, at first furnished with inconspicuous patches of white fibrils from the universal veil (especially near the margin), at times faintly canescent over all, usually variously streaked or with irregular watery spots, viscid, color "cinnamon" over all but becoming a dark, dull brown (nearly "cinnamon-brown"); flesh moderately thick and brittle, "pale pinkish buff," watery and darker (near "cinnamon-buff") in the stipe, odor and taste mild; lamellae very pale dull brown when very young (definitely not white), almost concolorous with the pileus in age, adnexed, close but distinct, moderately broad (5–6 mm. ±), edges nearly even; stipe 3–5 cm. long, 10–15 mm. thick, equal above the flanged basal oblique bulb, solid, pallid within, exterior whitish and silky overall, cortina white and scanty, universal veil scanty, bulb 2 cm. ± broad and pointed below; spores ellipsoid, slightly roughened or practically smooth, sordid ochraceous tawny under the microscope in KOH, 7–9 × 4–5 μ; basidia four-spored; cheilocystidia and pleurocystidia not differentiated; pileus-trama with a distinct but thin gelatinous pellicle, the hyphae 3–5 μ thick and hyaline, the floccose tissue immediately beneath the pellicle made up of hyphae with pale cinnamon-brown contents, the remainder of the flesh hyaline.

In arcs or clumps in an oak-hickory woods, Ann Arbor, Sept. 15 (*A. H. Smith 15379*—TYPE); Sept. 16 (*15411, 15399*); and Sept. 28, 1940 (*15546*).

Cortinarius squalidus is very similar in its characters to *C. allutus* Sec. sensu Lange, but from the available information cannot be referred to it. Henry has divided that species into two varieties, var. *lutea* and var. *rufescens*. *C. squalidus* is never bright yellow in any part so it cannot be referred to the former, and it does not become reddish in age or when dried so it cannot be referred to var. *rufescens*. *C. squalidus*, as its name indicates, is a very dull brown fungus presenting a rather sordid appearance in all except

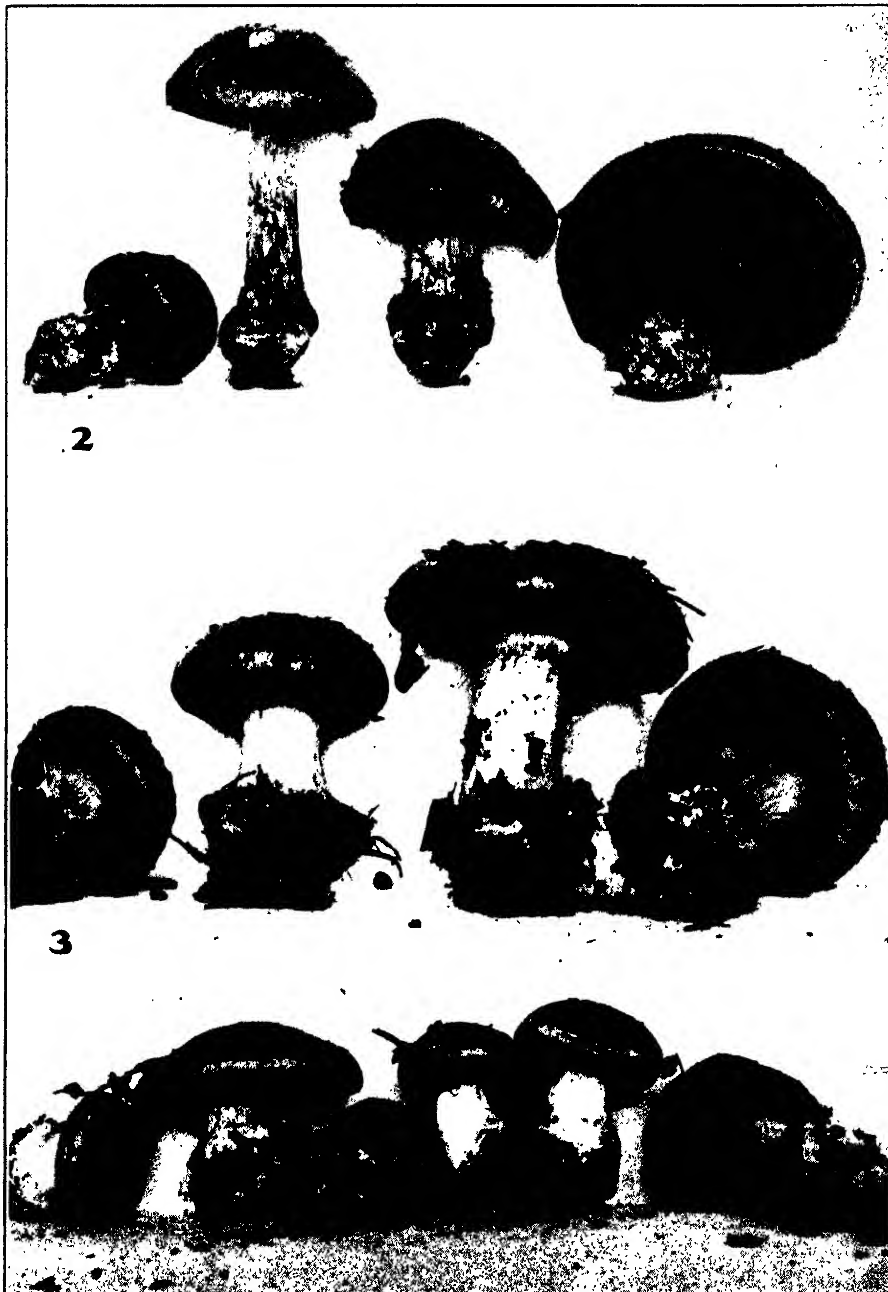

FIG. 2. *Cortinarius subsolitarius* Smith. × 0.7. FIG. 3. *Cortinarius citrinipedes* Smith × 0.7. FIG. 4. *Cortinarius squalidus* Smith. × 0.7.

the button stages. *C. nappus* as described by Henry (1) is apparently close but has spores 9–12 × 5.5–6 μ and is generally a more robust species. In addition, its colors tend to be yellow or yellowish in places.

Cortinarius subsolitarius Smith, sp. nov. Figure 2. Pileus 4–6 (10) cm. latus, obtusus, demum subumbonatus vel planus, glaber, virgatus, obscure violaceus, demum subolivaceo-violaceus; lamellae obscure violaceae demum brunneo-tinctae, angustae, adnatae; stipes 3–5 cm. longus, 10–15 mm. crassus, obsolete bulbosus, obscure violaceus; sporae 7–9 × 4 μ.

Pileus 4–6 (10) cm. broad, obtuse, becoming obtusely umbonate to nearly plane, the margin remaining incurved for a long time, surface viscid, glabrous but conspicuously streaked or blotched or with irregular watery spots, occasionally with fibrils along the margin, color "dull bluish violet (2)" the watery streaks darker (very dark violet), sometimes "dark dull bluish violet (2)," developing a distinct greenish gray tinge in age and fading only slightly, sometimes yellowish on the disc in extreme age; flesh thin, "pale campanula-blue" but becoming more greenish blue in age, "dark dull bluish violet" and watery punctate in the stipe, concolorous with the pileus in age, taste mild, odor faint and earthy, no color changes noted on bruised portions; lamellae "dark dull bluish violet (2)" and remaining so a long time, in unopened buttons "dusky blue-violet," in age merely tinged cinnamon-brown from the spores, close, adnate but tending to become adnexed, narrow to moderately broad, edges crenulate; stipe 3–5 cm. long, 10–12 mm. thick at the apex, up to 20 mm. at the base, solid, bulb marginate at very first but stipe soon merely clavate and hardly flanged at the base, concolorous with the gills and silky, cortina at first "dark dull bluish violet" fading only slightly; spores 7–9 × 4 μ, subamygdaloid, rusty brown under the microscope in KOH; basidia four-spored; cheilocystidia scattered, cylindric, hyaline, 28–39 × 4–6 μ; pleurocystidia not differentiated; gill-trama pale sordid vinaceous in KOH, especially near the gill-edges; pileus-trama homogeneous beneath a gelatinous pellicle, the hyphae of the pellicle pale yellowish in KOH, a region of yellowish brown floccose tissue immediately beneath the pellicle.

Scattered to gregarious on humus in oak-hickory woods, Ann Arbor, Sept. 11 (*A. H. Smith 15307*), Sept. 16 (*A. H. Smith 15377*—TYPE), and Sept. 16, 1940 (*15400*).

I have collected occasional specimens of this species in the vicinity of Ann Arbor for the past ten years and referred them doubtfully to *C. aggregatus* Kauff. because of the small spores. During September, 1940, both *C. subsolitarius* and *C. aggregatus* were found, the latter very abundantly, and a comparison of fresh specimens was made. The outstanding single feature of *C. subsolitarius* is the very deep, heavy violaceous persistent color of all its parts. As the fruiting bodies age, a greenish cast develops but the color in general does not become appreciably lighter. In a few old pilei yellowish spots developed on the disc and the flesh was yellowish around the wormholes. The bulb is very small and soon obsolete, being about as inconspicuous as that of *C. corrugatus*. *C. aggregatus* fades to a much paler color, has a

larger bulb and occurs in great quantities. In all of the specimens of *C. aggregatus* which I have seen, the colors of young individuals were paler than those of *C. subsolitarius*. *C. glaucopus* and its forms have the small spores but lack the uniform dark violet shades and show a very decided change of color from youth to old age. All the *C. glaucopus* which I have collected dried a greenish to rusty yellow over the pileus. Specimens of *C. subsolitarius* dry either dull violet or dull violaceous brown.

NEW OR REDESCRIBED SPECIES OF PHLEGMACIUM

CORTINARIUS CLARICOLOR Fr. sensu Ricken.? Pileus 5–9 cm. broad, convex to obtusely umbonate, nearly plane in age, the margin often remaining slightly decurved, sometimes repand and wavy or scalloped, surface glabrous and viscid, at times decorated with scattered fibrils along the margin, color "ochraceous tawny" (yellowish brown) over the disc and "warm-buff" (very pale buff-yellow) along the margin, sometimes with a canescent appearance as in *Pholiota caperata;* flesh thick and firm, whitish to faintly yellowish, unchanging, taste mild, odor fragrant when young pilei are cut; lamellae at the very first with a caesious tinge, soon pallid or "avellaenous" and finally pale "clay-color," close, 2–3 tiers of short individuals, broad (8–12 mm.), bluntly adnate at first, becoming adnexed in age; stipe stout, 8–10 (12) cm. long, 10–18 mm. thick at the apex, clavate-bulbous, the bulb 2.5–4 cm. thick, solid, texture cottony in the interior, whitish or faintly yellowish within, surface evenly colored like the context and densely silky from the copious cortina, with pale yellowish subannulate bands over the lower portion in age from the broken universal veil, u. veil white at first but remnants slowly lutescent, in age the flesh becoming slightly rusty brown around the wormholes or the surface brownish where handled; spores 9–11 (12) × 5–6 (7) μ, dark rusty brown under the microscope in KOH, coarsely tuberculate; basidia four-spored; cheilocystidia and pleurocystidia not differentiated; pileus-trama with a thick gelatinous pellicle, the hypae 4–5 μ thick and yellowish in KOH.

Gregarious on humus in low or upland oak woods, Sharron Hollow, and Ann Arbor, Sept. 14 and 16, 1940 (*15361, 15418*).

From his comments (2) it is quite clear that Kauffman's collections from New York, on which he based his report, represent some other species, which one I am not prepared to say. The small spores, subemarginate bulb and lack of a universal veil do not allow his collections to be placed here. In my specimens the white-fibrillose remains of the universal veil showed a tendency to become yellowish in age. This is not in accordance with the European concepts. The bands on the lower part of the stipe were never as highly colored as in *C. luteoarmillatus*, however. The gills of the younger specimens in all of my collections, as well as on all of the material I observed growing in the woods, consistently showed a caesious tinge. Many European authors described the young gills as pallid and slowly becoming brownish. Konrad and

Maublanc (4, pl. 126) give the spores as 11–15 × 6–8 μ, a rather serious discrepancy in this genus and indicating that their species is not that of Ricken.

Because of the zones on the stipes of old specimens some might be tempted to refer my material to *C. triumphans*. In my collections of the latter, however, the universal veil was distinctly colored in button stages and the pileus was also brighter.

Cortinarius luteoarmillatus Smith, sp. nov. Pileus 3–5 cm. latus, obtusus, demum convexus, viscidus, glaber, ad marginem fibrillosus, pallidus vel albido-argillaceus; lamellae sordide albidae demum argillaceae; stipes 5–6 cm. longus, 8–15 mm. crassus, clavatus, deorsum luteo-armillatus, sursum albidus et albido-fibrillosus; spores 8–10 × 5–6 μ.

Pileus 3–5 cm. broad (all rather young), obtuse, the margin incurved, becoming broadly convex as they mature, surface viscid, glabrous, with scanty remnants of a cortina along the margin, spot-like scales developing on the disc, color "cinnamon-buff" when young, "pinkish buff" in age (pale brownish buff) the disc usually shaded a bit darker than the margin; flesh thick, firm, hard, odor faintly fragrant, taste subnauseous, no color changes when cut or bruised; lamellae moderately close, adnexed, narrow (all young) 2–3 tiers of short individuals white to "tilleul-buff" (whitish), slowly becoming sordid brownish, edges even; stipe 5–6 cm. long, 8–15 mm. thick at the apex, clavate bulbous, (2 cm. thick at the base), rounded below and furnished with white rhizomorphs, the lower enlarged area with delicate yellowish zones of fibrils from the remains of a universal veil, cortina white and copious, apex loosely silky-fibrillose and appearing white because of the veil-remnants, solid and whitish or pale buff within; spores 8–10 × 5–6 μ, ellipsoid, nearly smooth, very pale yellowish brown under the microscope in KOH; basidia four-spored; cheilocystidia and pleurocystidia not differentiated; pileus-trama with a gelatinous pellicle made up of hyphae 3–4 μ thick and nearly hyaline when mounted in KOH.

Gregarious on muck soil in low woods, Sharron Hollow, Sept. 14, 1940 (*A. H. Smith 15360*—TYPE).

This species appears to be well characterized by the yellow zones of fibrils distributed over the lower third of the stipe, the whitish gills and very pale buff pileus along with the small spores. Were it not for the yellow zones left on the stipe by the universal veil, I should be inclined to identify my collection with *C. lustratus* Fr. or *C. sebaceus*. The spores and gelatinous pellicle of the pileus exclude *C. albidifolius* Pk., a species with apparently the same stature as *C. lutcoarmillatus*.

C. triumphans has similar but darker yellow bands on the stipe, but is readily distinguished by the colors of both pileus and gills. There appear to be several forms very similar to *C. triumphans* in North America. During the past season I collected one with spores 7–9 μ long and 6–8 μ wide which may be a distinct species. Its gills were pale caesious at first as in typical *C. triumphans*. My material, however, was not sufficient for a critical study.

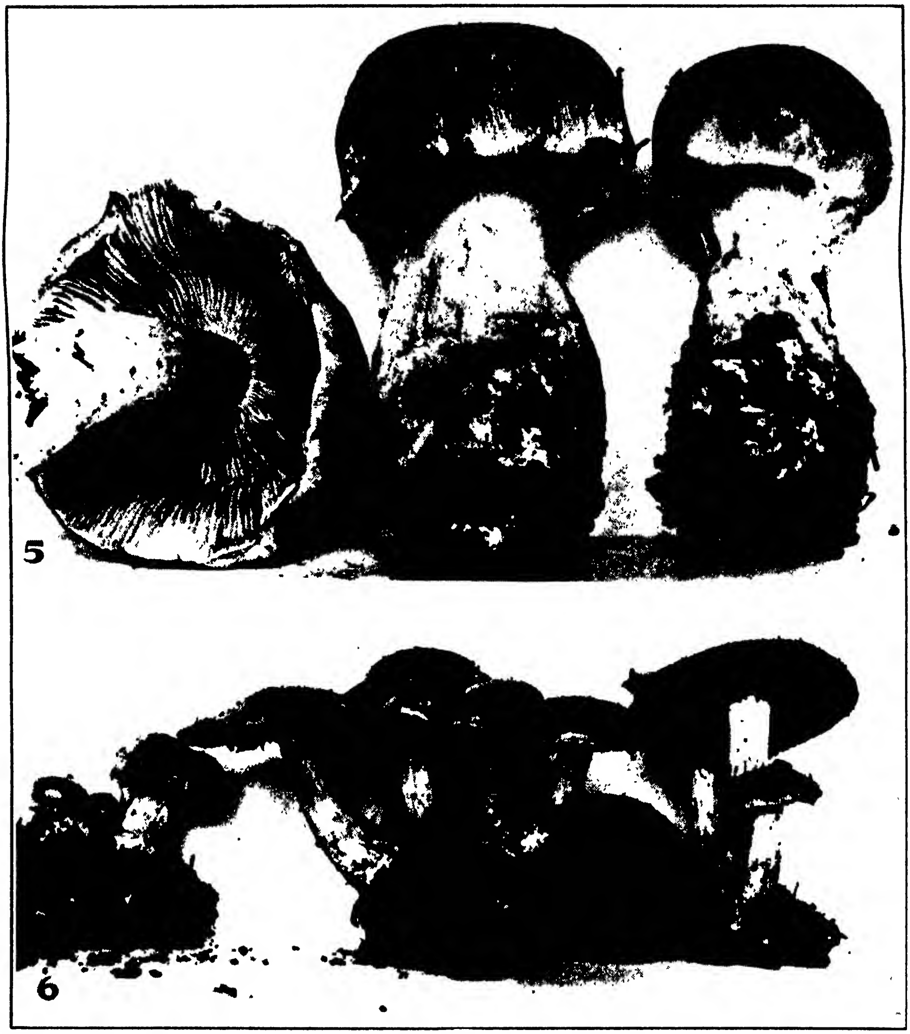

FIG. 5. *Cortinarius claricolor* Smith. ×0.7. FIG. 6. *Cortinarius fumosifolius* Smith ×0.7.

NEW AND REDESCRIBED SPECIES OF INOLOMA AND TELAMONIA

CORTINARIUS OBLIQUUS Pk. Pileus 7–10 cm. broad, very broadly convex, the margin decurved, becoming plane with an arched margin or the margin slightly uplifted thus creating a broad shallow central depression, surface dry and appressed silky fibrillose, the margin fringed or with adhering fibrillose patches, color "pale vinaceous fawn" (pallid with just a tinge of flesh-color) in age becoming darker and near "vinaceous buff" (darker pinkish brown); flesh thick and firm, "pallid purple-drab" in the stipe and along the gills, taste mild, odor fragrant; lamellae narrow (7–8 mm. in caps 10 cm.

broad), close to crowded, 2–3 tiers, depressed adnate to adnexed, with a decurrent tooth in age, "dark vinaceous brown" (dull purplish red) in youngest specimen (no buttons available), becoming dark cinnamon brown at maturity, edges eroded; stipe 7–12 cm. long, 1–2.5 cm. thick above the almost marginate bulb (2–3.5 cm. broad), solid, pallid purplish within, surface densely silky fibrillose from the remains of the cortina, toward the apex "light heliotrope-gray," (pale purplish gray—with only a tinge of vinaceous), pallid downwards, with a short sheath of matted fibrils formed by the universal veil remnants and terminating at the margin of the bulb; spores 8–10 (11) × 5–6.5 μ, ellipsoid, tuberculate, dark rusty brown in KOH; basidia four-spored, more or less sordid vinaceous brown when revived in KOH; cheilocystidia and pleurocystidia not differentiated; gill-trama and pileus-trama homogeneous.

Gregarious under beech, La Badie Lake, near New Hudson, Oakland Co. Sept. 23, 1940 (*15438*). The above description was taken from this collection.

The form described here differs so much in general proportions from typical specimens of the species that it seemed best to give a complete account of it. Large fruiting bodies such as this are known to occur in other species of Cortinarius and also in other genera of agarics. This is the second time I have encountered this particular form of *C. obliquus* in Oakland County, Mich. It was found first near Milford on Aug. 23, 1937. Both collections were made during dry weather following a period of very heavy rainfall.

Cortinarius subtestaceus Smith, sp. nov. Figure 7. Pileus 3–7 cm. latus, obtusus vel convexus, demum subplanus, saepe subdepressus, siccus, innate fibrillosus, pallide testaceus demum obscure testaceo-brunneus; caro aquose brunnea, demum pallida; lamellae argillaceae demum ochraceo-brunneae, subconfertae, latae, adnatae; stipes 6–14 cm. longus, 10–15 mm. crassus, clavatus, deorsum testaceo-cinctus, sursum sericeus et pallidus, solidus; sporae 9–11 (12) × 6–7 μ.

Pileus 3–7 cm. broad, obtuse to convex, expanding to plane or broadly convex, sometimes with a low umbo, surface dry and densely innately fibrillose under a lens (appearing glabrous to the naked eye), in age the fibrils arranged in small patches (as seen under a lens) but not becoming scaly, occasionally rimose over the disc in extreme age, margin delicately fringed with fibrils at first, color evenly "fawn-color" when young (rather bright vinaceous brown), becoming darker and near "walnut-brown" in age (full vinaceous brown); flesh watery brownish (pinkish buff) soon pale pinkish buff or pallied (a bit more vinaceous brown in the stipe), firm, fairly thick, taste mild, odor faintly acidulous; lamellae "clay-color" to near "buckthorn-brown" in unopened buttons (sordid yellowish brown), becoming "ochraceous tawny" at maturity, (tawny-brown), close but becoming nearly subdistant, 68–76 reach the stipe, 1–2 tiers of short individuals, moderately broad (6–7 mm.), broadest near the stipe, adnate, becoming shallowly adnexed; edges even; stipe 6–14 cm. long, 10–15 mm. thick at the apex, enlarged below to a narrowly clavate bulb and tapered to a point at the base, sometimes nearly fusiform or in age nearly equal and somewhat rooting, lower one third to one half covered by bands of "army-brown" to "fawn-

color" universal veil remnants, cortina white and silky and the upper half of the stipe whitish to pallid because of a coating of appressed, silky fibrils; spores 9–11 (12) × 6–7 μ tuberculate, ellipsoid, pale yellowish brown under the microscope in KOH; basidia four-spored; cheilocystidia none; gill-trama brownish-cinnamon when revived in KOH; flesh of pileus homogeneous and paler than the gill-trama.

Gregarious on humus in low woods, La Badie Lake, Oakland Co., Sept. 24 (*A. H. Smith 15458*—TYPE), and Ann Arbor, Sept. 28, 1940 (*15548*).

FIG. 7. *Cortinarius subtestaceus* Smith. × 0.7.

The surface of the pileus in this species has a dry appearance, but the flesh is watery punctate and changes color appreciably when it fades. Consequently the fungus is placed in Telamonia along with *C. armillatus*. Although the descriptions of these two read somewhat alike, one would never confuse the species in the field. *C. armillatus* is very common in northern Michigan, where I have collected it on numerous occasions. Its veil remnants are a much brighter red, its stature is much more robust, the stipe much thicker at the base, and its spores are much darker brown when mounts of both in KOH are compared under the microscope. The most conspicuous difference, however, is in the color of the universal veil. At first I was inclined to consider the collections cited above as belonging to *C. haematochelis*. Bresadola has given the length of the spores of that species at 7–8 μ long, and, judging by his illustration, its universal veil is colored more like that of *C. armillatus*. Some consider *C. haematochellis* as a synonym of *C. armillatus*.

C. paraguadis also has a somewhat similarly colored though poorly developed universal veil, but its spores are smaller, its stipe is equal and becomes dark purple at the base. Both *C. paraguadis* and *C. armillatus*, according to my experience, grow in conifer or mixed conifer and hardwood forests. *C. subtestaceus* was found on low ground under ash, elm, bur-oak and soft maple. The only conifers within a radius of one hundred yards were larch trees well out in the bog. *C. veregregius* Henry apparently has the same type of veil as *C. subtestaceus*, but its gills are described as brighter yellow and its spores are smaller and not ellipsoid.

Cortinarius fumosifolius Smith, sp. nov. Figure 6. Pileus 2–3.5 cm. latus, convexus demum subplanus, albo-fibrillosus, subglabrescens, obscure purpureo-brunneus, hygrophanus demum pallide argillaceus; lamellae fumosae, demum argillaceae vel sordide alutaceae; stipes 2–3 cm. longus, 4–6 mm. crassus, bulbosus, fibrilloso-volvatus, cavus, dense albido-fibrillosus; sporae 7–9 × 4–5 μ.

Pileus 2–3.5 cm. broad, broadly convex or with a flattened disc at first, in age broadly convex or with a low obscure umbo, surface appearing dry and whitish because of a dense covering of loose white fibrils, in age the fibrils becoming aggregated into patches scattered over the entire surface, margin ± fringed at first, color evenly "benzo-brown" (dull purplish brown) beneath the fibrils, the surface moist, hygrophanous and fading to sordid "pinkish buff," usually remaining more or less covered by the white fibrillose patches in age; flesh thickish, fragile, watery, "benzo-brown" but soon fading to pale sordid buff, odor and taste not distinctive; lamellae "light drab" (brownish gray) when young, sordid "clay-color" in age (pale alutaceous), adnate, becoming slightly adnexed, often with a decurrent tooth, close, 40–44 reach the stipe, 1–2 tiers of short individuals, moderately broad (3–4 mm.); stipe short, 2–3 cm. long, 4–6 mm. thick at the apex, base with more or less of a rounded bulb, fragile, hollow, watery, universal veil very copious and adhering to the bulb forming a volva-like fringe around it, appressed-silky above the bulb and with a whitish sheen, tinged violaceous brown beneath the fibrils (nearly concolorous with but paler than the pileus), cortina moderately copious; spores 7–9 × 4–5.5 μ, ellipsoid, dark rusty brown in KOH under the microscope, tuberculate; basidia four-spored; cheilocystidia and pleurocystidia not differentiated; gill-trama homogeneous, cells pallid in KOH; pileus-trama with a differentiated surface-layer of inflated cells 15–30 μ thick (tangential sections) which give a pseudoparenchymatous appearance similar to that in most species of Psathyrella, beneath it the tissue compact and floccose and made up of narrower hyphae, universal veil remnants resting on this layer and made up of narrow filamentous hyphae.

Gregarious under rhododendrons, Arboretum, Ann Arbor, Sept. 7, 1940 (*Mr. and Mrs. Paul Marshall Rea and A. H. Smith 15266*—TYPE). Known only from the type locality.

This *Telamonia* has so many unusual characters that it is hard to believe that it has not been previously described. The gray gills, the practically

marginate bulb (and development similar to that of species of Bulbopodium), the copious universal veil and the structure of the pileus are outstanding in this subgenus. The surface of the pileus is covered by universal veil remnants. These consist of hyphae 6–10 μ thick and with cells 50–100 μ long. The cuticle of the pileus is composed of pale brownish hyphae radially arranged and with the cells 15–30 μ thick and 30–60 μ long. This type of organization of the surface-layer in a Cortinarius is unique. I have encountered a rather large series of species in Inoloma which have marginate bulbs and a development comparable to that of the Bulbopodia, but only one other in Telamonia, and that one is still unidentified. More than likely *C. fumosifolius* was introduced. It is one of the few agarics I have collected on the compost around the rhododendron plantings in our local arboretum.

University of Michigan Herbarium
Ann Arbor, Michigan.

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INDEX TO AMERICAN BOTANICAL LITERATURE

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Papers that relate exclusively to bacteriology, forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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Beginning with this issue the Index to American Botanical Literature is classified under 8 headings as follows: Plant Taxonomy and Floristics (exclusive of fungi); Morphology (including anatomy, and cytology in part); Plant Physiology; Mycology and Phytopathology; Genetics (including cytogenetics); Ecology and Plant Geography; General Botany (including biography). It is realized that some articles do not fall readily in any of these classifications, and users of the Index interested in a particular topic are requested to examine also classifications which may include closely related topics. The editor and bibliographer will welcome suggestions and criticisms on the usefulness of the new arrangement.

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HETEROCARYOTIC VIGOR IN NEUROSPORA[1]

B. O. DODGE

Races of *Neurospora* normally develop certain structures which are characteristic of species of this genus. There are the orange-colored monilioid conidia, the incipient ascocarps, the microconidia, and finally, when two races of opposite sex are grown together, the ascocarps with asci. Races *C4* and *C8* of *N. tetrasperma,* for example, which will be mentioned many times in this report, produce all the structures just mentioned. They are of opposite sex in their reactions. Race *C4* produces a fluffy aerial growth in Difco potato dextrose agar tube cultures. The conidia tend to be of a rosy pink color. On the same medium race *C8* produces fewer conidia and much less aerial growth. The conidia of *C8* are bright yellowish-orange in color.

HOMOCARYOTIC AND HETEROCARYOTIC MYCELIA

When two races of *N. tetrasperma* which are of opposite sex are grown together there is a rapid migration of nuclei through the openings at the points of anastomoses (Dodge 1935a, 1935b). Some years earlier the writer (1930, 1931) realized that a nuclear migration through the openings in the cross-walls of the radiating hyphae must occur to account for the peculiar distribution of ascocarps (1930a, fig. 3). Actual proof of such a migration was not forthcoming until later (1935a). If races *C4* and *C8* are grown in a plate culture the nuclei of race *C8* migrate over to the *C4* side by way of the small openings in the cross-walls (fig. 1). Even though the cells of races *C4* and *C8* may contain from one to several nuclei each, these nuclei are all haploid and in each race all exactly alike genetically. Each race is therefore *homocaryotic.* After nuclei of the *C8* race have migrated to mingle with the *C4* nuclei in a common cytoplasm, however, the cells of the mycelium contain two different kinds of nuclei, and this part of the mycelium is now *heterocaryotic.* The growth from such a mycelium and from many of the conidia (fig. 2) produced on the new growth will also be heterocaryotic.

A NEW DWARF NON-CONIDIAL RACE OF NEUROSPORA

As the result of X-ray treatment or of natural somatic mutations we now have races of *Neurospora* which produce few, if any, monilioid conidia. Otherwise such "non-conidial" races may be perfectly normal.

Recently we have obtained a new non-conidial race, one that does not seem to produce any incipient ascocarps. Furthermore, it is a dwarf race

[1] Paper presented at the meeting of the National Academy of Science, held in Madison, Wisconsin, October 13–15, 1941.

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which grows so slowly that when transplanted to a fresh medium growth is first evident only after two or three days. Later the growth rate increases somewhat. It usually requires a week or more for the mycelium to cover an area 2.5 cm. in diameter. A culture in a Kolle flask will grow across from one side of the flask to the other, a distance of 9.5 cm., in about four weeks at room temperatures. This race will be referred to as "*Dwarf 16.*"

In tube cultures a week or ten days old, the growth at the lower part of the slant takes on a bright lemon- or cadmium-yellow color, and the growth becomes more or less warty or nodular. At the top of the slant in

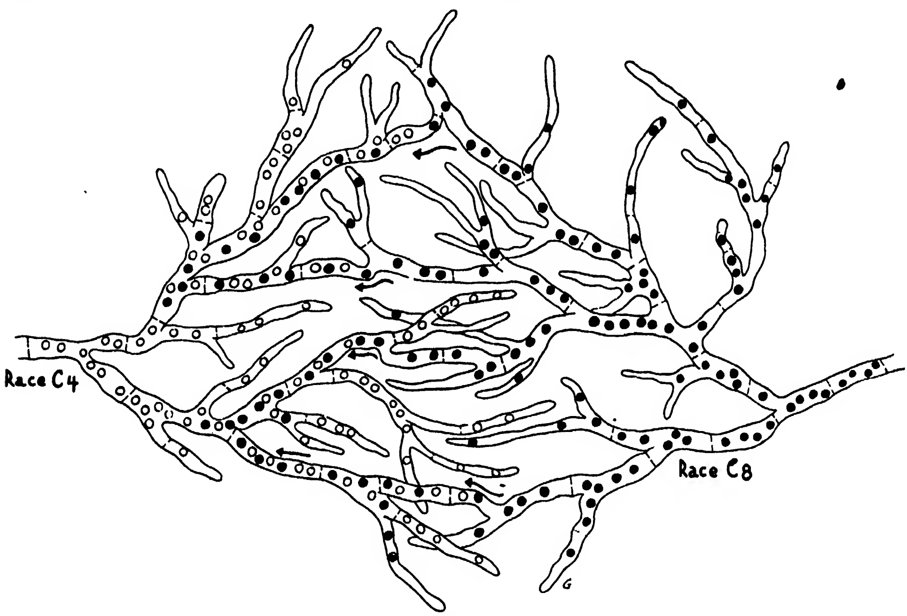

FIG. 1. Diagram showing how the nuclei in the mycelium of race *C8* (*Neurospora tetrasperma*) may migrate through the openings at the points of anastomoses to commingle with nuclei of race *C4* in a common cytoplasm. This part of the mycelium of race *C4* is thus changed from a homocaryotic to a heterocaryotic condition. The mycelium of *C8* has remained homocaryotic.

tubes or at the outer margin in plates, the aerial growth is somewhat fluffy and pale buff or only slightly yellowish.

Race *C8* usually grows a little faster than does race *C4*. When *C4* and *C8* are grown together in a plate the rate is about like that of *C8* alone. *C8* will grow 9 cm. in three or four days, depending on the temperature. It is apparent that both *C4* and *C8* grow many times as fast as does *Dwarf 16.*

SEX REACTION TYPE OF DWARF 16

In earlier work with races of *N. tetrasperma* carrying the so-called dominant lethal *I* (Dodge 1934) dwarf races were frequently obtained from

1-ascospore progeny. It was rarely possible to determine the sex or sex-reaction type of any of these dwarf races, which usually died after some weeks. However, if one allows the dwarf to grow alone for several days in culture before introducing the tester races a sex reaction may occasionally occur. The sex of *Dwarf 16* was determined by first allowing transplants to grow about four days in tube cultures of Difco potato dextrose medium. Races *C4* and *C8* were then used as testers, *C4* being introduced into one

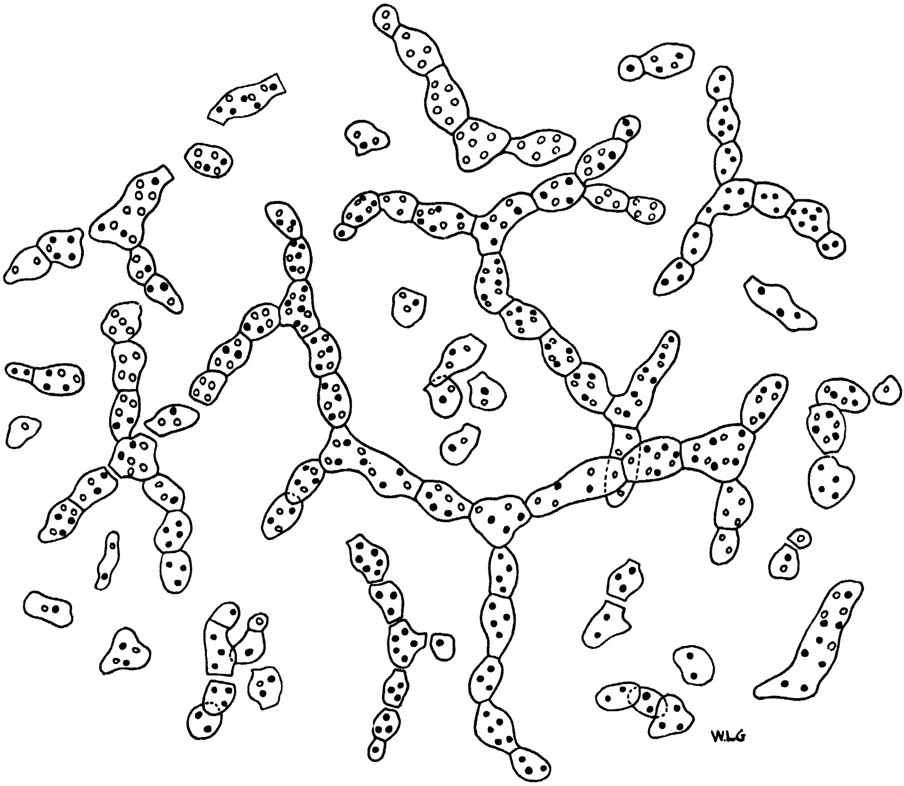

FIG. 2. Diagrams of monilioid conidia and hyphal fragments, the nuclear condition being purely arbitrary; some conidia are represented as homocaryotic, others as heterocaryotic. White and black used to distinguish differences in sex, or in some other factor pairs.

culture of *Dwarf 16*, and *C8* into another culture of *Dwarf 16*. The tube which now contained both *Dwarf 16* and race *C8* soon showed fluffy aerial growth and masses of yellowish-orange conidia were soon formed. Such large quantities of conidia are not formed in *C8* cultures under the same conditions. While numerous incipient ascocarps are always produced in cultures of *C8*, some of them being rather large, in the combination culture *Dwarf 16* + *C8* these incipient fruit bodies were more numerous, and they grew rapidly at first. They remained sterile, however, never producing asci.

Cultures of *Dwarf 16* to which transplants from a culture of *C4* had been added, also showed a rapid development of aerial growth and the production of excessive amounts of conidia which were more yellowish-orange than are conidia of *C4* when it is grown by itself. The incipient ascocarps developed rapidly, soon becoming fertile, discharging quantities of ascospores. *Dwarf 16* must, therefore, be opposite in sex to that of *C4* which we say is sex *A*, and it is of the same sex as *C8*, or sex *a*. *Dwarf 16* was also tested against another pair of wild-type testers, *S1* and *S9*, with the same results. By our usual methods of testing for sex reaction, *Dwarf 16* is unisexual, sex *a*.

DIFFERENT TYPES OF HETEROCARYOTIC MYCELIA AND CONIDIA INVOLVING DWARF 16

As noted previously, *Dwarf 16* produces few if any conidia by itself. The following questions arise: Do any of the conidia abstricted in the culture *Dwarf 16* + *C4* contain *Dwarf 16* nuclei in addition to nuclei of race *C4*? Are conidia which contain only *Dwarf 16* nuclei ever abstricted? Both questions may be answered in the affirmative. Proof was obtained at various times as follows, the results are considered here as a whole.

Thin sowings of conidia from a culture of the now supposedly heterocaryotic race *Dwarf 16* + *C4* were made on the surface of hard agar plates. The conidia were allowed to grow for twelve or more hours at 27° C. By this time growth from the larger conidia was rather extensive. The smallest conidia, however, showed only emerging germ tubes. Both kinds were isolated, but particular care was taken to select a number of the smaller later-germinating ones. Within two or three days, it was usually clear that of the 1-conidium isolates not just two or, at most, three types of cultures had been obtained; there were instead quite a number of different types.

First, there were many cultures which grew vigorously from the first, developing masses of deep cadmium-orange conidia and finally ascocarps with spores.

The aerial mycelial growth and the conidia produced in a few cultures were of a bright lemon-yellow color. Growth was vigorous, the color was certainly not that of *C4* by itself, and formation of ascocarps was so long delayed that we had hopes of discovering a startling cytoplasmic inheritance from the yellow dwarf. While such an effect is neither excluded nor proved as yet, in time these yellow cultures all produce ascocarps. Fertility was of a low grade, however.

Certain cultures of a third type were like typical *C4*, although the conidial masses were rather darker pink, bordering on orange in color. They never produced fertile ascocarps, and they reacted under tests as sex *A*, the same as race *C4*.

A fourth type of isolate was distinguished by its slow rate of growth and failure to produce very many conidia, at least not until after a week or ten days. They were described as dwarfish, but they were in no sense dwarfs like *Dwarf 16,* and no yellow color ever appeared. The surface mat of mycelium remained a very light pink. Transfers from such cultures sometimes produced more conidia, resembling more *C4* in this regard. Although cultures of this type all reacted as sex *A*, they are being studied further, as will be noted at another time. They may prove highly interesting.

A fifth type would include all dwarf isolates which finally became bright lemon-yellow and showed nodular growth at the base of the slant. They reacted as sex *a*, the same as *Dwarf 16*, which in fact they were, barring any cytoplasmic effects due to their association with *C4* nuclei in the same cytoplasm.

Finally there were in a sixth type isolates which during two or three weeks made very little growth beyond a few short hyphal branches. Some of these eventually grew enough to show that they were real *Dwarf 16* type, but a number died without making much growth.

In all, nearly 400 1-conidium isolates were obtained at various times from cultures in which *Dwarf 16* and race *C4* were growing together. Of this number at least 50 isolates proved to be real yellow dwarfs like *Dwarf 16*. Two hundred and eighteen (218) isolates sooner or later produced ascocarps with ascospores. The remaining isolates were recorded as producing conidia but no ascocarps with spores. All of this type that were tested proved to be of sex *A* reaction type like that of *C4*. A number of these conidial cultures showed a very decided yellowish cast or coloration of mycelia and conidia as though some cytoplasmic effect had been carried over because of a former association in the same cytoplasm with *Dwarf 16* nuclei. As long as such isolates were observed no fertile ascocarps were discovered, but further study would very likely prove that they were actually bisexual and thus contained also nuclei from the *Dwarf 16;* this would account for the yellow coloration.

THE QUESTION OF CYTOPLASMIC INHERITANCE RAISED

It is clear from the results given above that one can obtain conidia from a mixed culture, *Dwarf 16* + *C4,* which develop not only typical *C4* types, testing as sex *A,* but also conidia which developed yellow dwarf growth very like that of *Dwarf 16* which by itself abstricts few if any conidia. As further proof that the mycelium in the mixed cultures is actually heterocaryotic we find that ordinarily a large percentage of the isolated conidia give rise to a third type, vigorously growing fertile cultures producing ascocarps which mature ascospores. This would give us only three types of isolates, whereas, as noted previously, one can readily distinguish a number of dif-

ferent types of cultures. The reasons for this apparent anomaly may well be worth considering. The first point to be emphasized here is that nuclei from *Dwarf 16* race and those from race *C4* can, at will, be brought together in a common cytoplasm giving us a heterocaryotic mycelium. Furthermore, the two nuclear components or elements in such growth can be isolated and grown separately again.

If it is the *Dwarf 16* nuclei that originally migrated over into the cells of race *C4* mycelium, then one may well ask which race contributed the cytoplasm carried in the conidia which are abstricted and which carry only *Dwarf 16* nuclei. It is not at present clear from the evidence just how much such a cytoplasm-nucleus relation may account for the intermediate types of cultures which one may obtain when he starts with only two kinds of nuclei. The second type described above might be considered as showing a cytoplasmic effect.

There appear not only various grades of fertility (some cultures maturing ascocarps quickly, others only after many days) but gradations in coloration, amounts of conidia produced, and striking differences in vigor or growth rates among the isolates. Some of these differences must be due to the relative numbers of the two kinds of nuclei included in particular conidia isolated. The writer (1930b) has proved that microconidia of species of *Neurospora* will germinate, but very slowly, only after about two days. Hyphae from the germ tubes grow very slowly for several days. The mycelium is not that of a dwarf, however. Eventually the mycelia from microconidia become rather normal, so that matings can be made with them as with mycelia from the monilioid conidia or ascospores. The reasons for the very slow growth-rate at first are clearly the small size of microspores and the fact that they would contain only a single nucleus each. Tertiary conidia are very small and germinate slowly. In isolating single conidia from a mixed culture *Dwarf 16* + *C4,* one can with sufficient care pick out conidia which will give rise to *Dwarf 16* types. The germ tubes from such conidia develop a sort of distorted rosette type of branching. If the conidium is very small, germinating slowly but developing long branched germ tubes, a sex *A* reacting type of growth may be obtained. Some of these cultures are typical of race *C4,* while others certainly fail to develop as normal *C4* types. The cultures will, however, be unisexual, sex *A* in their mating reactions.

Large rapidly germinating conidia will usually give fertile cultures with ascospores. It has been shown (Dodge 1927) that the size of ascospores of species of *Neurospora* depends largely on the number of nuclei included in the spore at its origin. The great difference in the sizes of conidia and hyphal fragments is also due to a certain extent to the number of nuclei included at abstriction. Conidia formed on the heterocaryotic mycelium *Dwarf 16* + *C4* must vary not only in the kinds of nuclei they contain, but also in the

relative numbers of each kind. If a conidium should contain only a single nucleus of *Dwarf 16*, it would very probably be unable to develop much beyond the formation of a germ tube. A small ascospore containing only a single nucleus of a similar dwarf race might well be able to grow into a good dwarf because of the much larger amount of cytoplasm in the ascospore. Whether the difference in the number and kinds of nuclei in conidia accounts wholly for the differences in types of growth, may be questioned, however. This material certainly should be studied from the standpoint of the effects of the cytoplasm, as well as the possibility that *Dwarf 16* contains of itself two kinds of nuclei.

NUCLEAR MIGRATIONS BETWEEN RACES OF THE SAME SEX

Up to now the writer has been inclined to the view that the nuclear migrations proved to occur in cultures of *N. tetrasperma* are due to sex attractions, or to sex hormones, or to the attractions of "opposites" (which may or may not be the same things). While "protoplasm" may be seen streaming from cell to cell and through openings where hyphae anastomose, there is no proof that nuclei are carried along in the stream. It would seem strange if nuclei of two races of the same sex, but differing in other genetic factors could migrate to mingle in a common cytoplasm. From the experiments now to be described it can be said that other forces than sex attractions do cause nuclei to migrate freely from hyphae of one race into the cells of another race which is of the same sex reaction type.

Aerial growth in mixed cultures, *Dwarf 16* + race *C8*, soon shows much more vigor than does the mycelium in cultures of *C8* alone. Many more conidia are also formed at the crown of growth. These conidia are usually a bright yellow-orange in color. Furthermore, the incipient ascocarps are very numerous and they increase rapidly in size, at first resembling young ascocarps which develop in cultures in which races of opposite sex are being grown. Usually incipient ascocarps of race *C8* grow very slowly at first, only a few of them ever attain the size of fertile fruit bodies. In cultures of *Dwarf 16* + race *C8*, as noted previously, the little bodies enlarge very rapidly from the first, but after attaining fair size, cease further development. So far as we have been able to discover, none of them ever produces asci.

In a mixed culture, *Dwarf 16* + *C8*, with a commingling of nuclei from both races in a common cytoplasm, the cells would be homocaryotic for sex, but heterocaryotic for certain other pairs of factors represented in the two kinds of nuclei. *Dwarf 16* has the genotype *aDc*, while race *C8* has the genotype *adC*.[2] Two methods were employed to prove that such heterocaryotic mycelia and conidia were present in the mixed cultures, *Dwarf 16* + *C8*. These experiments will be described below.

[2] *A*, *a* are sex reaction factors; *C*, *c* are factors governing production of conidia; *D*, *d* factors for deliquescent ascus abortion (Dodge 1935; Dodge & Seaver 1938).

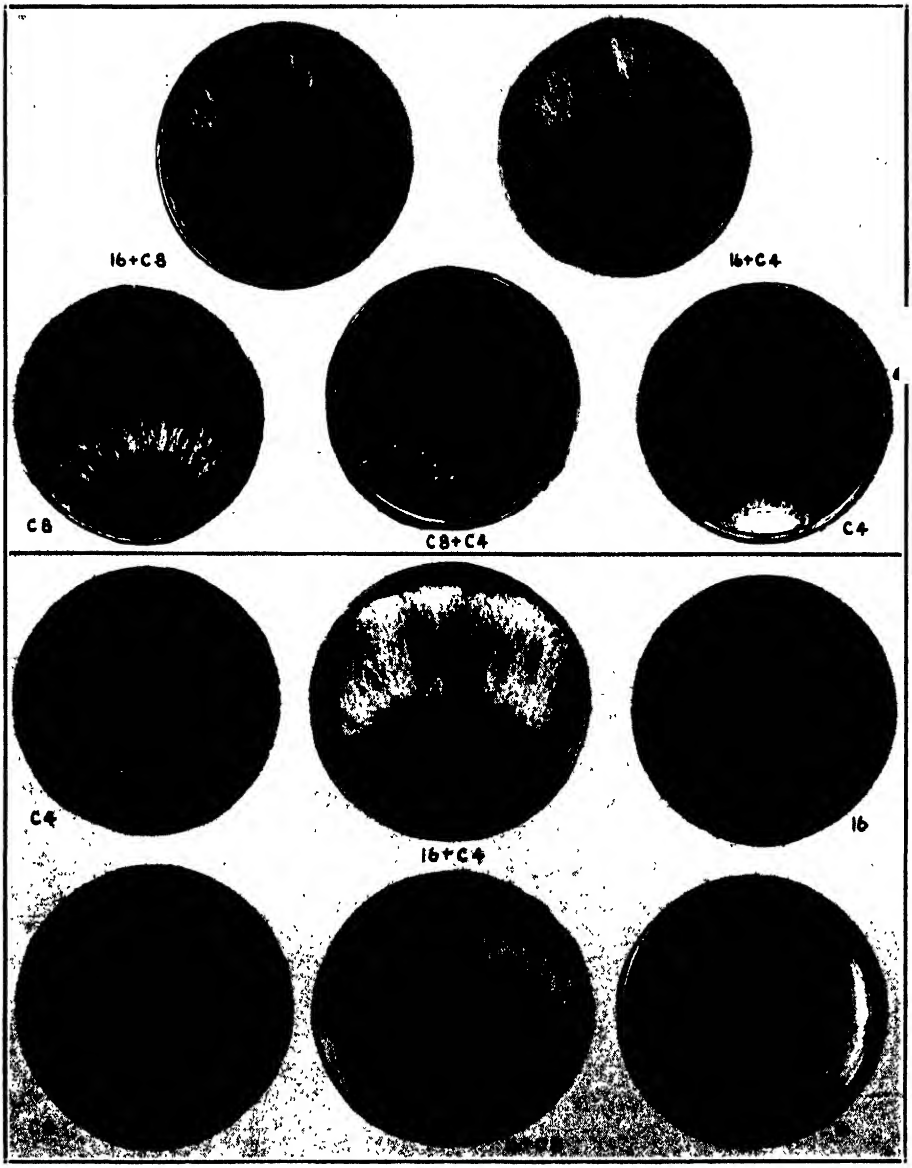

FIG. 3. Above: Photographs of cultures 24 hours old in plates 9 cm. in diameter and incubated at 30° C. The mycelium of the two heterocaryotic races, *Dwarf 16 + C8* and *Dwarf 16 + C4*, have completely covered the plates while that of race *C4* is not yet half way across. The heterocaryotic race *C8 + C4* has grown about the same distance as has race *C8*. Below: Comparative amounts of growth in plate cultures at room temperatures for 34 hours. *Dwarf 16* has made no visible growth in either culture. The mycelia of races *C4* and *C8* have not grown half way across as yet, while the heterocaryotic mycelia have just about covered the plates.

INCREASED VIGOR INVOLVING RACES OF OPPOSITE SEX

As noted above, when the *Dwarf 16* was grown in culture with race *C4* which is of the opposite sex, mixed cultures showed not only a more vigorous growth but also larger quantities of conidia than are ever developed in culture of *C4* or *C8* alone. The differences in the growth rates of these three races are much more readily demonstrated in plate cultures. If one transplants some mycelium of race *Dwarf 16* into one plate, some conidia of race *C4* into a second plate, and some conidia or mycelium from the mixed culture *Dwarf 16* + *C4* into a third plate, and allows growth to proceed for from 24 to 40 hours, depending on the temperature, it will be seen that little or no growth is evident in the first plate, *Dwarf 16,* at the end of this time. (It requires about two days for growth to be apparent.) Mycelium of race *C4* would have grown nearly half way across the plate while the heterocaryotic mycelium, *Dwarf 16* + *C4,* would have grown completely across the plate (fig. 3). Similar tests repeated many times show that the heterocaryotic mycelium grows from two to three times as fast as does that of race *C4* alone. Growth by the dwarf for the first day or two, as noted above, is negligible.

A very striking demonstration of these different growth rates may be obtained by using U-tubes. In this case the potato dextrose agar medium should be prepared by using tap water instead of distilled water and the medium should be sterilized intermittently in a steam sterilizer at about 92° C. This is to avoid driving the air from the agar in the U-tubes.[3] If distilled water is used and the medium is autoclaved, there is very little oxygen left in the agar, so that even though, as Denny (1933) has shown, only a minute quantity of oxygen is necessary for growth, the mycelium will make little headway down into the agar. With the agar prepared as noted above, one may inoculate the agar in one arm of a U-tube with mycelium of *Dwarf 16,* the agar in one arm of a second tube with *C4* and a third tube with the heterocaryotic conidia of *Dwarf 16* + *C4.* At the end of a week's time it may be seen that the surface of the slant in the tube containing *Dwarf 16* is nearly covered with the mycelium of the dwarf which has just begun to turn yellowish at the center. Race *C4* will usually have grown down through the agar and appeared in the other arm of the tube and begun to produce some of the pale pink conidia in both arms. In the third tube containing *Dwarf 16* + *C4* the mycelium would have grown around through the agar very quickly and begun to produce large quantities of brightly colored yellowish-orange conidia. If one watches the growth of

[3] Since this paper was presented Beadle & Tatum (Proc. Nat. Acad. **27**: 409–506. 1941) have described a much better method for observing and measuring growth rates. They use horizontal tubes 13 cm. inside diameter and about 40 cm. in length. The ends are bent at a 45° angle. The medium is poured in to fill the tube half full. Our U-tubes were much larger in diameter.

the mycelium in the three tubes it will be very evident that the heterocaryotic mycelium has grown very much more rapidly than that of race *C4* and produces a great many more conidia than are formed by the *C4* race alone.

The great increase in vigor illustrated in figure 3 occurred when the medium was inoculated with conidia or with fragments of mycelia which contained two kinds of nuclei, *Dwarf 16* and *C4;* that is, when the transplanted mycelia or conidia were already heterocaryotic. This increased vigor may also be demonstrated when one inoculates a plate first with mycelium from *Dwarf 16* and then at a point nearby with conidia of race *C4*. Other plates may be inoculated, one with *Dwarf 16,* and the other with race *C4* alone for comparison. Up to a certain time there is very little difference between the amount of growth in the *C4* plate and that in the plate containing the mixture *Dwarf 16* and *C4*. Then it will be seen that the mycelium of the mixed culture is growing faster than is the mycelium of race *C4,* and by the end of the first or the second day, depending on the temperature, increased vigor is very evident. That the mycelium in the mixed culture has now become heterocaryotic may be proved by plating out the conidia formed at the outer margin. The smaller, more slowly growing conidia isolated will develop either the yellow *Dwarf 16* or pinkish growth much like that of *C4*. The mycelia from the larger, more rapidly growing conidia eventually produce fertile ascospores. Intermediate types of growth will be obtained because of quantitative differences in the nuclei included in the conidia isolated. This point will be discussed below.

INCREASED VIGOR INVOLVING RACES OF THE SAME SEX REACTION

It was stated above that in the mixed cultures in which *Dwarf 16* (sex *a*) and race *C8* (sex *a*) were grown, the aerial growth was more vigorous and it produced more conidia than was evident in the culture containing race *C8* alone. By inoculating three plates as before, one with *Dwarf 16,* the second with race *C8,* and the third with mycelium or conidia from the mixed culture of *Dwarf 16* + *C8,* it was shown that the mycelium of the mixed culture grew very much more rapidly than did the mycelium in the culture *C8*. As a rule, race *C8* grows somewhat faster than does race *C4*. The heterocaryotic mycelium[4] *Dwarf 16* + *C8* on the other hand grows about as rapidly as does heterocaryotic mycelium *Dwarf 16* + *C4*. When race *C4* is grown with race *C8* in a mixed culture so that a heterocaryotic mycelium *C4* + *C8* is developed, it has been shown that the latter mixed culture, the heterocaryotic race *C4* + *C8* does not grow appreciably faster than does *C8* alone. In some tests race *C8* alone grows the faster. In fact, not infrequently in tube cultures a mixed culture, race *C4* + *C8,* produces fewer

[4] See below for proof that this mycelium is actually heterocaryotic.

conidia than does race *C4* alone. It is well known that race *C8* seldom produces on our potato dextrose agar very many conidia. By using U-tubes as described above for demonstrating increased growth of the heterocaryotic mycelium *Dwarf 16* + *C4* the great increase in vigor and in the production of conidia by *Dwarf 16* + *C8* can be emphasized.

Race *C8*, when grown on Difco potato dextrose agar media is characterized by the paucity of conidia and the tendency to form amorphous blotches or patches (Pease 1936) on the surface of the culture. These patches later turn a sordid reddish brown color which gives the culture the appearance of being badly contaminated with yeast and bacterial colonies. This effect is well brought out in U-tube cultures. The patches are not prominent at first in plates, being covered by the fluffy mycelium, which later collapses. When, however, nuclei of *Dwarf 16* come into the same cytoplasm with nuclei of this race *C8* a most beautiful transformation takes place. No sordid amorphous blotches now mar the surface of growth. Instead, a vigorous mycelial growth develops on the surface and soon a crown of bright yellowish-orange or cadmium-yellow conidia appears.

More accurate determination of growth rates was later obtained when small blocks of agar bearing fresh mycelial growth were used as transplants. When conidia alone are used there are bound to be errors because recently formed conidia germinate more quickly than do old or dried conidia. If it is necessary to transplant conidia, one should allow growth to proceed for a number of hours and then begin taking measurements from the limits of growth at the end of this preliminary period. At 30° C. it was found for example, in one set of plates, that starting with transplants of blocks of agar, race *C4* grew approximately 3 cm. during the first 24 hours, while race *C8* grew 4.1 cm. and race *C4* + race *C8*, heterocaryotic grew only 3.8 cm. During the same period heterocaryotic races *Dwarf 16* + *C4* grew 8.2 cm.

PROOF FOR MIGRATION OF NUCLEI OF THE SAME SEX

To avoid confusion, a detailed description of the two sets of experiments by which it was proved that two genotypically different kinds of nuclei of the same sex may commingle in a common cytoplasm was postponed in order to emphasize the main feature of this paper, namely, increased vigor of certain heterocaryotic mycelia over that shown when the two components in each case are grown separately. The first experiments involved race *Dwarf 16* and race *C8*. Sowings of conidia were made as described above. Germinating conidia were isolated, great care being taken to isolate the smallest, more slowly growing conidia. The mere fact that one could in this way obtain 1-conidium cultures of what appeared to be pure race *C8*, would mean nothing, since *C8* produces its own conidia. But if a considerable number of the small isolates grew to become yellow dwarfs like *Dwarf 16* this would indi-

cate perhaps that just as in the mixed culture of *Dwarf 16* + race *C4*, conidia are cut off bearing only *Dwarf 16* nuclei, in spite of the fact that the dwarf produces few if any conidia by itself. Of the 1-conidium isolates obtained from the mixed cultures, *Dwarf 16* + *C8*, 15 grew to become good yellow dwarfs that could not by tests be distinguished from *Dwarf 16*. Eighteen grew to look and react like pure *C8*. There were several somewhat different types of cultures which grew vigorously from the start; many of them were bright lemon-yellow or cadmium-orange in color. None of these ever produced fertile ascocarps, neither did transfers from them. The isolate cultures that were entirely unlike either pure *Dwarf 16* or race *C8* indicated that in such cases nuclei from *Dwarf 16*, as well as nuclei from race *C8*, had become associated in these conidia because of nuclear migrations, just as if two races of opposite sex were grown together.

Other sowings of conidia from cultures of *Dwarf 16* + *C8* were made, care now being taken to select the larger, more quickly growing conidia. Most of the isolates grew vigorously and produced masses of highly colored conidia. None of these cultures produced fertile ascocarps. Some 30 of them were then grown separately with both *C8* and *C4* testers. No ascocarps with asci were produced in cultures with *C8*, but in all cultures with *C4* fertile ascocarps were matured. The critical point in these tests was to find two entirely different kinds of ascocarps in some of the fertile cultures. If the isolated condia contained both *Dwarf 16* and *C8* nuclei which are of the same sex reaction type, sex *a*, then nuclei from the inserted tester *C4* could join with either of the two kinds of nuclei, *Dwarf 16* or *C8*, to bring about fertilization. Both *C4* and *C8* races carry a certain recessive lethal "*d*" (Dodge 1935) for deliquescent ascus abortion. Asci homozygous, *dd*, for this lethal would abort. Such asci would arise if nuclei from *C8* (sex *a*) joined with nuclei from *C4* (sex *A*). But if nuclei of *Dwarf 16*, sex *a*, joined with nuclei from *C4*, then asci with spores would be found in fertile ascocarps because the nuclei from *Dwarf 16*, carry the wild type *D* allele of *d*. Asci being heterozygous *Dd* would produce spores.

It was easy to distinguish these two kinds of ascocarps in many of the mixed cultures because on Difco potato dextrose agar medium at the temperatures then prevailing many of the aborting asci become indurated, dark brown in color, and devoid of ascospores. This feature is very helpful because of certain additional sterility factors operating in these complex hybrid asci, often lead to ascus abortion even though the asci are heterozygous *Dd*, and ordinarily become indurated. Furthermore, some of the very numerous ascocarps in these cultures would mature at least a few spores. There was a sufficient number of cultures in which all asci in some ascocarps produced a full quota of spores, while all asci in other ascocarps in the same culture produced only indurated asci with no spores, to prove that a heterocaryotic

mycelium may often arise through nuclear migrations involving nuclei of the same sex.

Another experiment was carried out to prove this point. As a result of crossing a certain vigorous non-conidial race with race *C4* a bright yellow vigorously growing race, *Race 6,* was obtained. This race reacted with race *C4* to produce fertile ascocarps, but when grown with race *C8* the mixed cultures remained sterile. Conidia from these mixed cultures (*C8* + *6*) were isolated. None gave fertile cultures. Again 1-conidium isolates were made as before, but from an isolate culture this time. The new isolates were grown with *C4* and with *C8* separately. None of the cultures with *C8* (sex *a*) produced fertile ascocarps. In many cultures with *C4* two kinds of ascocarps were easily distinguished. One kind produced many asci, all of which aborted and usually became indurated without producing any ascospores. This would indicate that they arose from the cross *C4* + *C8*. Other ascocarps produced some asci with spores, showing that these arose from the cross *C4* + *6*. Since race No. 6 is *acD*, and *C4* is *ACd,* the asci would be heterozygous for all three pairs, including *Dd,* and some spores would be delimited. The asci from this cross would show a great lack of fertility, however.

HETEROCARYOTIC VIGOR AND HYBRID VIGOR DISTINGUISHED

When it is recalled that *Dwarf 16* produces few if any conidia, it is all the more clear that increased growth and the production of such large quantities of conidia in mixed cultures, *Dwarf 16* + *C8,* must be due to the combined effects of bringing together in the same cytoplasm the two kinds of nuclei, *Dwarf 16* and *C8*. The same conclusion may be drawn from the effects visible when *Dwarf 16* and *C4* are grown together. This appears to be a condition or a situation which has not been reported previously. One may grow two different races of a fungus, races either of opposite or of the same sex reaction, separately in cultures under controlled conditions where the growth rates may be studied carefully and the production of conidia noted. Then, at will, one can bring the nuclei of the two races together in a common cytoplasm, with the result that the heterocaryotic mycelium grows with such astonishing vigor and produces conidia in such great quantities. Since this vigor is expressed in connection with heterocaryotic structures this type of vigor is being described as ***heterocaryotic vigor*** to distinguish it from the true hybrid vigor manifested by diploid organisms such as our industrial yeasts or by higher plants or animals. What is the meaning of this heterocaryotic vigor? Is it at all comparable, for example, to hybrid vigor in corn? Can anything be learned about hybrid vigor in general by a careful study of heterocaryotic vigor in *Neurospora*?

Robbins (1941) in a recent paper on hybrid vigor (heterosis) in tomatoes

has suggested that hybrid vigor may be due to the ability of the heterotic hybrid to synthesize a full quota of vitamin-like growth substances, whereas the parents of the hybrid were not able to do this. He has suggested to the writer personally that perhaps a somewhat similar explanation might account for heterocaryotic vigor, modifying the explanation to cover the haploid organization of the structures involved.

On this basis one could say that *Dwarf 16* is able to synthesize adequate amounts of, say, vitamins or growth substances 1, 2, 3, and 4, but be unable to synthesize enough of 5, 6, 7, and 8. On the other hand, race *C4* (or race *C8*) may not be able to synthesize enough of vitamins 1, 2, 3, and 4, but be able to produce an adequate supply of 5, 6, 7, and 8. When nuclei of the two races are brought together in a common cytoplasm, the amounts of growth substances now synthesized by one of the components of the heterocaryotic mycelium supplement the amounts of growth substances synthesized by the other component. The mycelium now fortified strongly by an optimum of vitamin-like growth substances manifests a great increase in vigor, and excessive amounts of conidia are produced.

In crossing *Dwarf 16* and race *C4* a number of new dwarf races have been obtained, races which are self-sterile, but which cross readily with both testers *C4* and *C8*. Whatever the results of further studies of *Dwarf 16* and the new dwarfs should be, the main point at issue here will not be affected, namely, that heterocaryotic mycelia containing nuclei from both *Dwarf 16* and either race *C4* or race *C8* show greatly increased vigor of growth and increased production of conidia over either *C4* or *C8*.

This factor complex concerned with increased heterocaryotic vigor is heritable. Certain new dwarf races, such as race *Dwarf 130,* one of the unisexual progeny of the cross *Dwarf 16* × *C4*, has much the same morphological and physiological characters as *Dwarf 16*. When nuclei from *Dwarf 130* mingle with nuclei of either *C4* or *C8* heterocaryotic vigor is manifested.

The new dwarf races which remain sterile when grown alone, but which fruit with both testers *C4* and *C8* are of great interest. Just how so many of the ascospores from which they were derived could have contained two nuclei of opposite sex at their origin, and at the same time both nuclei carry factors for dwarfness, is not easily explained even on the basis of errors due to random selection. If *Dwarf 137*, for example, while remaining self-sterile, is able to produce ascospores with spores when grown with *C4* but only ascocarps with deliquescent aborting asci with *C8*, it may be possible to separate the two components, providing that these components can be grown by themselves. Instead of being a dwarf this race *137* should really show heterocaryotic vigor, unless neither of its components is able to synthesize adequate amounts of certain growth substances.

INDIVIDUAL HOMOCARYOTIC INHERITED VIGOR

In previous discussions we have distinguished between heterocaryotic vigor expressed by structures whose cells contain two or more different kinds of haploid nuclei, and true hybrid vigor expressed by diploid structures whose nuclei are actually diploid. A third type of vigor is shown by certain *Neurospora* races obtained by crossing *Dwarf 16* with race *C4*. When one mates these two races the only real hybrid structures obtained are the asci with their fusion nuclei. Reduction is followed by a third division which provides each ascus with eight haploid nuclei. In the obligately heterothallic species, such as *N. crassa*, eight ascospores are then formed. The ascus of *N. tetrasperma* normally contains four spores which have two nuclei each at their origin. These nuclei are usually of opposite sex reaction. Occasionally an ascus has five ascospores. In this case two of the spores are small and contain only one nucleus each at their origin. By selecting these small ascospores one obtains unisexual races. Several hundred new races, haploid progeny of *Dwarf 16* + race *C4*, were obtained by selecting ascospores at random. No attempt was made to isolate only the small unisexual ascospores; the slowness of germination and the irregularity of the germ tubes insured the selection of a large percentage of new dwarf races. While this work has not been carried on sufficiently to warrant drawing any conclusions, it may be said that a number of dwarf races have been obtained. These all remain sterile when grown alone but only a very few of them react as unisexual races when tested against races *C4* and *C8*. In many cases the new dwarfs fruit with both *C4* and *C8*, which proves conclusively that they are actually bisexual, although self-incompatible.

Recently Dickson (1939) reported that he crossed two races of *N. crassa* and obtained a segregant which he thought showed hybrid vigor because it grew more vigorously than did either of the races crossed. On further consideration he questioned this idea, because the new race did not show at certain temperatures much if any increase in vigor over the parent races at those same temperatures. Dickson should have realized that even if his new race had shown a greater growth rate than the parents at all temperatures he could not have called it hybrid vigor because his new race was not a hybrid originally. A race of *N. crassa* arises from a single ascospore which is provided at its origin with a single haploid nucleus. Even though the cells of the mycelium may contain from one to several nuclei each, these nuclei are all haploid and they are all exactly alike genotypically. Since the mycelium of Dickson's race was not diploid it could not very well have shown true hybrid vigor under any conditions of growth whatsoever.

The heterocaryotic vigor which is described here is in quite a different category because the cells of the mycelium in each instance contain at least

two genotypically different kinds of haploid nuclei, regardless of whether the two kinds are of the same or of opposite sex in their reactions. A number of the new races grew very vigorously from the start and produced large quantities of conidia without ever developing ascocarps with asci. Numbers of these races were tested for their sex reactions and were found to react with only one of the two tester races, which shows that they were unisexual. Since neither of the parents, *Dwarf 16* and race *C4*, is a vigorous race, it is clear that the vigor now shown by these new races must be due to a recombination of factors governing growth and production of conidia. On the hypothesis that vigor of growth depends upon the ability of the race to synthesize adequate amounts of growth substances, individual vigor must be distinguished from heterocaryotic vigor, because, in the case of individual vigor, the genes or factors concerned with the synthesis of growth substances must be included in a single haploid nucleus of the ascospore giving rise to the new race. Even though true hybrid vigor, heterocaryotic vigor and the individual vigor, just mentioned, may be due to exactly the same or similar causes, they should be distinguished because they are often manifested in quite different ways; they are expressed in connection with particular structures and at particular times in the life cycle of the organisms concerned.

Dickson (1939, p. 127) refers to his earlier work (1935) on growth rates of various segregant races of *Coprinus sphaerosporus*, claiming that certain haploid segregants showed hybrid vigor. He was not referring to dicaryons as manifesting "hybrid" vigor. He distinctly says this of his haploid segregants. Dicaryotic vigor might very well be expressed when the nuclei of two haploid segregants are brought together in the same cytoplasm. Dickson did not, however, study growth of dicaryons as compared with that of the individual components. Such a study would no doubt have borne fruit. After rereading the papers referred to above and a third paper by Dickson (1936) one must be convinced of the necessity for distinguishing heterocaryotic vigor, and individual haploid segregant vigor from each other and both from true hybrid vigor. If we do this we shall avoid confusion, we shall be better understood and, what is more important, our students will think more clearly when we are discussing growth, reproduction and inheritance in the fungi.

SUMMARY

A new yellow non-conidial dwarf race, *Dwarf 16,* which by itself grows very slowly, about 2 cm. per week, reacts as a unisexual race, sex *a,* when grown with the tester races *C4* and *C8* of *Neurospora tetrasperma.* When this dwarf is grown with race *C4,* which is of the opposite sex, sex *A,* nuclear migrations occur so that nuclei of both races come together in a common heterocaryotic mycelium, which shows a great increase in vigor of growth and production of conidia. The new mycelium grows two or three times as fast as

does that of race *C4*. The same phenomenal increase in the growth rate and production of monilioid conidia occurs when *Dwarf 16* is grown with race *C8*, which is of the same sex reaction, sex *a*. This increased vigor is referred to as *heterocaryotic* vigor and it is distinguished from individual haploid segregant vigor and from true hybrid vigor which is expressed in connection with diploid organisms. It is suggested that perhaps the hypothesis offered by Robbins to account for hybrid vigor in tomatoes, for example, may be applied here. That is, the growth substances synthesized by *Dwarf 16* supplement those synthesized by race *C4* (or *C8*) so that the heterocaryotic mycelium has an optimum of those vitamin-like substances that control growth.

Preliminary work in crossing *Dwarf 16* with race *C4* shows that this factor complex is heritable. Some of the haploid segregants carry in turn very similar factors for heterocaryotic vigor.

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STEM ROT OF TUBEROUS BEGONIA

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INTRODUCTION

Within recent years the tuberous begonia, *Begonia tuberhybrida* Voss, has become a popular and valuable floricultural crop in California. The plant is well adapted to both garden and glasshouse culture, particularly in coastal, temperate areas. In the summer of 1938 the writer's attention was called to a disease causing the collapse and death of tuberous begonia plants in several large commercial glasshouses in the San Francisco Bay region. Later the same trouble was found in commercial plantings at Capitola, California. Upon examination of diseased material it was found that the malady was due to a fungous infection. The causal fungi were identified as *Pythium intermedium* de Bary and *P. ultimum* Trow and their pathogenicity demonstrated in inoculation tests. It is the purpose of this paper to discuss the symptoms of the disease, the causal organisms, host range, and certain control measures.

REVIEW OF LITERATURE

Species of *Pythium* have previously been reported as pathogens of *Begonia* spp. In 1925 Braun (1925) reported that *Pythium debaryanum* Hesse, *P. debaryanum* var. *pelargonii* Braun, and *P. splendens* Braun were capable of infecting an unidentified species of *Begonia* by inoculation. The writer considers *P. debaryanum* var. *pelargonii* synonymous with *P. debaryanum*. Pape (1927) in Germany stated that leaf cuttings of the Gloire de Lorraine begonia (*B. socotrana* Hook × *B. dregei* Otto & Dietr.) were destroyed in the propagation bench and that *P. debaryanum* was probably implicated; he later (1933) confirmed this report and added that this and other *Pythium* spp. were the causes of a seedling disease. Flachs (1931) also mentions *P. debaryanum* as the cause of a seedling disease of *Begonia* spp. Sharples (1930) reported a natural infection of an unidentified species of begonia in Malaya by *P. splendens;* no description of the disease was given. Swift (1932) in New York described some control measures for a leaf and stem rot of *B. semperflorens* Link & Otto caused by *P. debaryanum*. According to Dr. B. O. Dodge,[2] Swift's fungus was later identified by Charles Drechsler as *P. ultimum*. In 1938 (Middleton *et al.*, 1938) a disease

[1] Grateful appreciation is expressed to Professor M. W. Gardner, Division of Plant Pathology, University of California, for advice and assistance and the use of controlled temperature facilities.

[2] In correspondence with the writer, Nov. 22, 1938.

affecting the roots, crown, basal portion of the stem and leaves of *B. semperflorens* was reported as occurring both in California and Missouri; the causal agents were designated as *P. debaryanum, P. splendens,* and *P. ultimum.* Subsequently the writer (Middleton 1938) presented a preliminary report of natural infection of tuberous begonia by *P. intermedium* and *P. ultimum,* and also described a stem rot of *B. lloydii* (horticultural name) caused by

FIG. 1. Stem rot of tuberous begonia: A, non-inoculated control; B, *s* symptoms produced on young tuberous begonia plant by *Pythium intermedium* 16 days after inoculation in the greenhouse. The lesion is sunken and vertically disposed, consuming a large portion of the stem. Identical symptoms are produced by *P. ultimum.*

P. ultimum. This was followed by a report (Nance 1939) that a *Pythium* sp. was responsible for a stem rot of tuberous begonia in New York and New Jersey. A culture of the latter fungus was obtained from Dr. A. W. Dimock and identified as *P. ultimum.*

SYMPTOMS OF THE DISEASE

The stems of naturally infected plants exhibit light brown, watersoaked lesions which are not usually sunken but occasionally may be. These lesions are vertically disposed and vary somewhat in size, though generally from 5 to 28 mm. in length and 3 to 8 mm. in width (figs. 1 A, B, 2). Lesions often coalesce, encompassing the stem, in advanced stages of the disease. Occasionally petiole and lamina infections have been observed. Petiole symptoms

resemble those of diseased stems; infected laminas have irregularly defined watersoaked areas which turn brown and become flaccid, the entire blade eventually collapsing.

Natural root and tuber infections have seldom been observed. When roots and tubers are infected they are discolored, watersoaked, and later pass into a flaccid state.

FIG. 2. Stem rot of tuberous begonia: *X*, Natural stem infection due to infection by *Pythium intermedium*. This type of darkened, not usually sunken lesion is most common and is usually associated with a leaf axil; this symptom is usually produced about 7 days following infection. Identical symptoms are produced by *P. ultimum*.

Petal infection has also been noted to occur under conditions of excessive overhead irrigation; petal symptoms are comparable with the laminar symptoms presented above but may be confused with those caused by *Botrytis cinerea* Pers. These symptoms are easily differentiated only if *Botrytis* is sporulating.

THE CAUSAL ORGANISMS

The fungi, *Pythium intermedium* and *P. ultimum*, were readily obtained

in pure culture from diseased material by tissue plantings made on plain water agar. The use of this medium permitted isolations to be made from badly diseased material without complication by contaminants.

Morphology. Both organisms produce a copious amount of white cottony aerial mycelium when grown on cornmeal, oatmeal or potato-dextrose agars. *Pythium intermedium* displays a pulvinate habit of growth, whereas that of *P. ultimum* is arachnoid. The hyphae of both organisms are cylindrical, smooth, nonseptate when young, measuring 1.7–6.5 μ in diameter (mean 3.5 μ) becoming septate with age.

Sporangia are produced freely on the substrates used, being most numerous on potato-dextrose agar; extramatrical sporangia predominate over intramatrical ones. The sporangia of *Pythium ultimum* are borne singly and are spherical when acrogenous, measuring 15.3–27.6 μ in diameter (mean 20.3 μ). Occasional intercalary bodies are found which are limoniform and vary in size from 16.1 μ in width and 19.5 μ in length to 23.7 μ and 27.4 μ, respectively (mean 19.1 μ × 22.8 μ). The sporangia of *P. intermedium* are typically catenulate, though occasionally borne singly and then terminal. Catenulate sporangia are usually spherical, measuring 17.3–24.7 μ in diameter (mean 21.5 μ), often up to 13 in a series, sessile or separated by short stalks. Sessile sporangia are sometimes pyriform.

Transfers of mycelial fragments from four-day old pea-broth cultures produce an abundance of sporangia when placed in sterile distilled water. The production of a vesicle at the tip of a short evacuation tube and the subsequent liberation of zoospores was commonly observed in *P. intermedium*. Sporangia of *P. intermedium* were also seen to germinate by the production of one to three germ tubes. All attempts to induce zoospore formation in *P. ultimum* were unsuccessful, the sporangia germinating by the production of from one to five germ tubes.

Oogonia of *Pythium ultimum* are produced freely when the fungus is grown on plain water agar. Oogonia are largely intramatrical, acrogenous, spherical, with a smooth, thin wall, measuring 17.1–25.3 μ in diameter (mean 20.6 μ). Antheridia are invariably of monoclinous origin and single, arising immediately below the oogonium and adjacent to it, somewhat clavate, short, upcurved, the apex contacting the oogonial wall. Infrequently two antheridia may be applied to the oogonium, in which case one of them is usually of diclinous origin from a proximal neighboring hypha. A prominent penetration tube is generally discernible.

Mature oospores of *Pythium ultimum* are aplerotic (not filling oogonial cavity), spherical, with a smooth, thick wall. The contents are somewhat granular, containing a single reserve globule and often a subspherical refringent body. Oospores measure 13.0–21.4 μ in diameter (mean 16.7 μ), including the oospore wall which is approximately 2.3 μ thick.

No oospores have been observed in any of the isolates of *Pythium intermedium;* this is in accord with the description of the species given by de Bary (1881) in which it is stated that oospores are unknown.

Temperature Relations. The relation of temperature to growth of the mycelium was determined for four isolates of *Pythium intermedium* and four of *P. ultimum,* from both stems and leaves of tuberous begonia. The culture tubes (2.1 × 20 cm.) used and the procedure followed in this study are those previously described by Tompkins and Gardner, (1935). Each tube used is provided with a glass dam at the open end. Thirteen milliliters of Difco cornmeal agar (pH 6.0) are added to each tube and allowed to cool to a uniform depth with the vessel in a horizontal position; the dam prevents the escape of the melted agar.

The tubes were inoculated near the dam at the open end with a small square of potato-dextrose agar containing the mycelium of the fungus cut from 3-day-old Petri dish cultures. Inoculated tubes were left at room temperature for 24 hours, the extent of growth at the close of the period being indicated by a wax pencil mark on the tube; subsequent measurements were made from this point.

Three tubes of each isolate were placed in a horizontal position in controlled temperature chambers at intervals of 3°, from 1° to 43° C. The cultures were incubated for a 72-hour period. The average growth in millimeters over 24-hour periods at the various temperatures is presented in table 1.

TABLE 1

Mycelial growth of Pythium intermedium and P. ultimum on Difco cornmeal agar at various temperatures

Degrees C.:	Average growth in millimeters over 24-hour period														
	1	4	7	10	13	16	19	22	25	28	31	34	37	40	43
Isolate															
P. intermedium															
Stem	2	4	7	11	14	16	21	23	22	21	5	t*	0	0	0
Stem	1	4	7	9	13	16	20	23	23	21	2	0	0	0	0
Leaf	1	4	7	9	12	16	19	23	24	19	8	1	0	0	0
Leaf	2	4	7	10	13	16	20	24	23	20	2	0	0	0	0
P. ultimum															
Stem	0	1	4	8	14	18	26	28	34	32	19	16	2	t	0
Stem	0	1	6	10	14	17	23	26	32	32	16	12	1	0	0
Leaf	t	3	6	13	15	18	23	27	31	34	30	23	t	0	0
Leaf	0	1	3	9	14	17	23	29	33	34	32	17	1	0	0

* t = trace.

The minimum temperature for growth of *Pythium intermedium* is approximately 1°, the optimum from 22° to 25°, and the maximum 34° C.; the minimum temperature for growth of *P. ultimum* is approximately 4°, the optimum from 25° to 28°, and the maximum 37° C.

While there are no recorded temperature relations of *P. intermedium* in the literature, the values given for *P. ultimum* are similar to those recently summarized (Tompkins *et al.* 1939).

Pathogenicity. Young tuberous begonia plants were grown in autoclaved soil in 6-inch pots. Pure cultures of *Pythium intermedium* grown on sterilized cracked wheat were added to the soil in one set and *P. ultimum* to that in a second set; to a third group was added sterilized cracked wheat. After from 14 to 20 days, 85 per cent of the plants in infested soil exhibited basal stem lesions. A few yellowed and wilted in the absence of any visible stem lesions; most of these were growing in pots infested with *P. ultimum,* although a few were observed to occur in pots in which *P. intermedium* was present. On removal these plants showed extensive root infection. Plants growing in soil containing only cracked wheat remained healthy.

Isolations made from artificially infected stems and roots proved pathogenic upon reinoculation, producing symptoms indistinguishable from natural infection.

In order to induce infection on the upper portion of the stem, probably naturally accomplished by zoospores and detached sporangia, small agar blocks containing the fungus mycelium were placed in the unwounded axils of the leaves and covered with sterile absorbent cotton saturated with sterile distilled water. After from 5 to 8 days infection was discernible. *Pythium ultimum* proved to be somewhat more pathogenic than *P. intermedium* when this technique was used. Isolations made from infected areas proved pathogenic upon reinoculation. No infection occurred when agar blocks devoid of fungus mycelium were similarly applied.

Leaf infection was obtained by placing agar squares containing the fungus on undetached and unwounded laminas. Plants so inoculated were placed in a moist chamber for 3 days and then removed to the plant bench. Two to 3 days later watersoaked areas developed which, upon isolation, yielded pure cultures of whichever fungus had been applied.

Further tests with both *Pythium intermedium* and *P. ultimum* demonstrated that stem and leaf isolates were equally pathogenic upon cross inoculation.

An experiment was devised to ascertain the ability of both *P. intermedium* and *P. ultimum* to cause damping-off of tuberous begonia seedlings. Two lots of five 6-inch pots each were filled with soil artificially infested with pure cultures of the fungi grown on cracked wheat; over this was placed one-quarter inch of sterile soil. Twenty-five seeds were then sown in each pot and the pots subirrigated. Two pots of soil mixed with sterile cracked wheat were similarly sown. Three weeks later the following survival counts were made: *P. intermedium* 53, *P. ultimum* 6, and checks (not inoculated) 202.

The pathogenicity of *P. intermedium* and *P. ultimum* to rooted cuttings

of *Begonia semperflorens* was determined. Using the soil and pot procedure described above, 50 pots each were inoculated. From two to three weeks later all the plants inoculated with *P. ultimum* showed root rot to the exclusion of any stem lesions as typified in inoculation experiments with *B. tuberhybrida*. All plants inoculated with *P. intermedium* were apparently healthy.

The pathogenicity of both *P. intermedium* and *P. ultimum* to stems and leaves of *Begonia lloydii* was conclusively demonstrated by employment of the agar block technique already given.

CONTROL

Since the causal organisms are soil fungi, the disease may be adequately controlled by soil sterilization. Proper precautions should likewise be taken to insure use of sterile pots and flats.

When potted plants are grown in close proximity to one another the disease assumes considerable importance. In such instances the disease is often distributed throughout the plot by means of leaf infections due to the overlapping and contact of these parts. Excessive irrigation is conducive to the disease, particularly when watered by an overhead sprinkling system. In order to overcome these difficulties a good cultural practice should be established and maintained. Such a program would necessarily include proper spacing of plants, moderation in watering, abolishment of overhead sprinkling practices and provision made for good aeration of glasshouse space. If the disease assumes epidemic proportions and it is deemed necessary to rely upon fungicidal control, copper-containing sprays such as ammoniacal copper, Bordeaux, Burgundy, or Cuprocide may be employed. Swift (1932) recommends soil applications of semesan.

SUMMARY

A stem rot of tuberous begonia, *Begonia tuberhybrida,* occurring in California, New York, and New Jersey is described.

The disease may attack plants of any size or age and is most severe under conditions of high soil and air moisture and from moderate to high temperature.

Symptoms of the disease consist of soft, unsunken or sunken, watersoaked lesions which are vertically disposed and may ultimately encompass the stem, causing the stem to collapse. Root, petiole, lamina, and petal infections are also reported.

The causal organisms are *Pythium intermedium* and *P. ultimum.*

The temperature-growth relations of the organisms were determined. The values for *P. intermedium* are: minimum 1, optimum 22–25°, maximum 34° C.; the values for *P. ultimum* are: minimum 4°, optimum 25–28°, maximum 37° C.

Infection of healthy tuberous begonias was obtained by artificial inoculation. *Begonia lloydii* was found susceptible to both causal organisms; *B. semperflorens* was susceptible only to *P. ultimum.*

The causal fungi were able to cause damping-off of tuberous begonia seedlings.

The disease may be satisfactorily controlled by using sterile soil, pots, and flats, good cultural procedures, and copper-containing fungicides.

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THE DEPENDENCE OF CERTAIN ANNUAL PLANTS ON SHRUBS IN SOUTHERN CALIFORNIA DESERTS

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For the plant physiologist one of the most intriguing problems of plant ecology is the question why a given plant grows in a given place. Certain aspects of this problem are under investigation and are fairly well understood. The distribution of plants is partly determined by climate, partly by soil, and partly by biotic factors such as seed distribution. But within a given set of climatic and soil conditions a large number of species can occur. The plant sociologist has grouped these species into well defined units which are termed plant communities, associations, etc. It is assumed that within each of these communities micro-climatic and soil conditions and survival values are such that the members of this particular community are favored over others so that the community as such persists.

It is not intended in this paper to examine critically the various factors which contribute towards the distinctness of a community. The survival values, mutual elimination, and similar Darwinian concepts are probably overrated in their influence on communities (see Kooper 1927). Besides, these factors, being only names for complexes themselves, are in great need of critical study before they can be accepted as a basis for explanation. Everyone who considers the fact that almost identical collections of plants are grown in scores of botanic gardens in widely different soils and climates is constrained to admit that the potential range of environmental conditions for many individual plants is far larger than the actual range of the communities in which such plants normally grow. Many assumptions have been made to bridge this gap between experience and theory, but most are more ingenious than likely.

Most natural plant communities are highly complex and the factors which result in the establishment of these communities include: climate, chemical and physical composition of soil, water, supply and germination of seeds, succession stages, history of previous vegetation, climate and soil conditions at the time of establishment of the community, etc. This complexity makes an analysis of the causal relations between the plants in a community well-nigh impossible. In a few cases, however, a clearer insight has been obtained by analyzing communities which occur under relatively simple conditions. A few papers of importance for our problem will be discussed in some detail.

The first paper is one by Kooper (1927), who studied the pioneer weed communities which appear immediately after plowing of the (mostly weedless) rice and sugar cane fields in the plains of Pasuruan in East Java. When

left fallow for several months these fields develop a rich vegetation of annuals. Within a limited area (10×10 m.) never more than 30 species (17 as a mean) out of a possible 300 weeds occurring in the whole region were found by Kooper to be growing together. The abundance of a single species did not change gradually, but the changes were very abrupt and were parallel for a number of species. In this way a limited number of weed communities is formed, each characterized by a fairly large number of constantly associated species. The limits of these communities are relatively sharp, so that one community with its typical constants suddenly changes into another community with a number of different constants over a distance of a few meters. One of the most remarkable facts found in connection with these communities was that their composition was already determined at the time of the germination of the seeds. In special germination experiments Kooper sowed sets of viable seeds in recently plowed soil normally carrying communities which lack these species. The results were definite. Only when the species sown normally occurred in the community would they germinate; otherwise no germination took place at all, and no seedlings developed. Thus the distinctness of the community is due to "birth control," which very definitely proves that these weed communities do not arise through competition and survival of the fittest.

Although association between the various members of one community was found, there were no correlations between the physical and chemical properties of the soil and the plant communities which grew on them.

All the observations of Kooper point towards the conclusion that these weed communities arise through a mutual influence of the plants on each other, and more specifically through influence of the previous vegetation on the germination of seed. This mutual influence cannot be due to physical factors, such as shading, since the nature of the communities is already determined at the time of germination. It seems highly probable, therefore, that there is a chemical inter-relation between the plants in one community which determines their germination and occurrence.

Critics of Kooper have questioned the general occurrence in Java of the phenomena studied by him. From personal experience, however, I can confirm his observations. In fallow rice fields near Semarang in the middle part of Java, with climate somewhat different from that of Pasuruan, I observed the same communities in which all constants occurred in the same relative abundance. This also is a good argument against the supposition that the composition of these weed communities is determined by climatic conditions.

In an investigation of the epiphytes in a tropical rain forest another type of mutual influence between plants was found (Went 1940). When the occurrences of the various epiphytic ferns, orchids, Ericaceae, etc., were noted in connection with the host tree on which they were growing, it was discovered

that certain epiphytes are found almost exclusively on certain host trees. This relation is so constant that trees can be identified by the epiphyte communities which they harbor. This relationship is apparently not due to physical properties of the host tree or microclimatic differences in the habitat which the various host trees offer. Therefore, we reach a conclusion similar to that of Kooper: namely, that the occurrence of one plant, the host tree, determines the presence of a certain number of other plants, epiphytes, through some unknown agent, presumably chemical.

If such distinct causal relationships between the members of communities exist, it becomes necessary to determine whether similar relations exist in other plant communities and whether the growing together of many plants is generally conditioned by mutual influences of these plants. Such influences are very hard to demonstrate wherever a continuous layer of vegetation occurs, since in that case it is practically impossible to determine (except experimentally) which plants are mutually dependent. In the desert regions, however, such inter-relationships should be much easier to investigate since their vegetation cover is not continuous and only the plants which are growing in groups together and which are separated from others by stretches of bare soil could be considered to influence each other. Therefore, a preliminary sociological study was made in the Colorado and Mojave deserts in Southern California during the spring of 1941.

In a number of localities occurrence of certain plants, mostly annuals, in connection with shrubs was noted. Different types of ecological analysis were tried, and finally the following one was adopted, being the simplest and giving the clearest results. All shrubs were counted distant 3 m. or less from a line followed on foot through the vegetation. In this way the shrub vegetation was recorded of a transect with the approximate dimensions of 400 × 8 m. The shrubs were classified in two ways; in the first place, according to their species; and in the second place, according to whether or not a certain annual was found in close association with the shrub in question. Results of such ecological analyses are brought together in tables 1, 3, 4, and 5. From these tables it can be seen that some annuals are closely correlated with certain shrubs and seem to avoid others. The shrubs which favor one annual do not always harbor certain other annuals, and vice versa. A very clear case of such vicarious occurrence is provided by *Caulanthus lasiophyllus* (H. & A.) Payson and *C. cooperi* (Wats.) Payson (see table 3).

By analyzing vegetation in this way it is soon found that a certain number of annuals bear no relation at all to any shrubs or other pre-existing vegetation. They are found predominantly in open spaces between shrubs, and generally these are the plants which form the marvelous mats of color which adorn our deserts in spring. They seem to grow equally well, or sometimes even better, in places where they have no direct contact with other

TABLE 1

Occurrence of six plants (annuals except Hilaria) with different shrubs in Borego Valley
Figures in brackets refer to plants growing with dead shrubs

	Encelia farinosa Gray (living)	*Encelia farinosa* Gray (dead)	*Franseria dumosa* Gray	*Hymenoclea salsola* T. & G.	*Hyptis emoryi* Torr.	*Dalea schottii* Torr.	*Larrea tridentata* Cov.	*Opuntia echinocarpa* Engelm. & Bigel.	*Krameria canescens* Gray	*Mirabilis bigelovii* Gray	No connection with any shrub	Total number of plants observed
No. of shrubs in one transect	248	84	172	136	51	46	12	13	8	3	.	772
Per cent shrubs harboring one or more specimens of the herbs												
Phacelia distans Benth.	30	(57)	50	68	47	20	7	46	62	100		352
Malacothrix californica DC.	5	(35)	27	15	14	4	25	31	12	0		125
Emmenanthe peduliflora Benth.	4	(36)	3	20	27	9	8	(31)	0	30		99
Rafinesquia neomexicana Gray	1	(11)	12	4	0	2	0	8	12	70	.	45
Hilaria rigida Thurb.) Benth.	2	(6)	8	4	(2)	0	0	0	0	0	..	38
Eschscholtzia minutiflora Wats.	0	0	0	5	(4)	4	0	(31)	0	0	......	22
Per cent distribution of each herb among the shrubs												
Phacelia	21	(14)	25	26	7	2	1	2	1	1	0	....
Malacothrix	9	(23)	37	17	6	2	2	3	1	0	0	. ..
Emmenanthe	10	(31)	6	27	14	4	1	(4)	0	1	2	....
Rafinesquia	7	(20)	47	13	0	2	0	2	2	5	2	..
Hilaria	13	(13)	34	13	(3)	0	0	0	0	0	24	.
Eschscholtzia	0	0	0	32	(9)	9	0	(18)	0	0	32	..

TABLE 2

Length of main stem and number of flowers of Rafinesquia neomexicana, growing in various shrubs in Borego Valley

	Number of specimens measured	Length in cm. (with extremes)	Number of flowers
In open, not with shrubs	4	16(10–25)	1.7
Unknown dead shrubs	5	19(15–25)	1.8
Dead *Encelia farinosa*	6	35(25–55)	5.0
Living *Encelia farinosa*	1	30	4.0
Dead *Opuntia echinocarpa*	2	25	3.0
Living *Opuntia echinocarpa*	1	60	17.0
Larrea tridentata	2	30(25–35)	3.5
Dalea schottii	4	45(25–65)	5.0
Franseria dumosa	10	55(35–70)	9.5

plants. In general, it can be said that the closer these plants grow together the smaller they remain. They are not more vigorous in the neighborhood of shrubs than they are in the open.

Many other plants are found both under or near shrubs and in the open. These plants may or may not show a preference for certain shrubs. In general in this group there is not a very large size difference between the free-growing plants and those growing near shrubs.

The third category, however, consists of annuals which are always found near shrubs. If such a plant has accidentally germinated away from any shrub, its development is extremely poor and its size is only a fraction of what it normally is. Examples of such plants are *Phacelia distans* Benth., *P. tanacetifolia* Benth., *Caulanthus cooperi*, *Rafinesquia neomexicana* Gray. In table 2 the sizes and number of flowers of the latter plant have been noted

TABLE 3

Occurrence of annuals in connection with shrubs growing in hills north of Kramer, Mojave Desert

	Total number of shrubs observed	Per cent shrubs harboring annuals		
		Caulanthus lasiophyllus (H. & A.) Payson	*Caulanthus cooperi* (Wats.) Payson	*Phacelia tanacetifolia* Benth.
Franseria dumosa Gray	60	15	60	75
Aster abatus Blake	40	10	10	5
Larrea tridentata Cov.	15	100	20	90
Lycium andersonii Gray	18	....	15	100
Grayia spinosa (Hook.) Moq.	16	60	30	100
Ephedra nevadensis Wats. (?)	3	50	100	80
Composite (*Tetradymia* ?)	11	....	60	....
Unknown dead shrub	3	100	100	....
Free, not connected with shrub	....	Many	Few	Very few

TABLE 4

Occurrence of Rafinesquia neomexicana Gray in connection with different shrubs. Approximate number of shrubs observed, total number of Rafinesquia present in those shrubs and percentage occurrence with each shrub noted for three different localities in the Eastern Mojave desert.

Name of shrub	Locality								
	North of Ludlow*			East of Ludlow			South of Amboy		
	No. of shrubs	No. of *Rafinesquia*	Per cent occurrence	No. of shrubs	No. of *Rafinesquia*	Per cent occurrence	No. of shrubs	No. of *Rafinesquia*	Per cent occurrence
Krameria canescens Gray	7	5	70	12	4	30	10	4	40
Franseria dumosa Gray	4	2	50	170	7	4	200	17	9
Hymenoclea salsola T. & G.	250	35	14	.....	.	.	...		
Larrea tridentata Cov.	50	6	12	100	2	2	100	0	0
Opuntia / Unidentified composites	500	20	4	......	...	....	25	0	0
Lepidium fremontii Wats.	....	....	...	......	...	...	100	0	.....
Dead plants	...	23	.	....	1	..	.....	2	
Ephedra	.....	..	..	.....	.	....	150	7	7
Free, not with shrubs	......	2	....	....	3	..	.....	1	...

* Observations by Mr. P. Gorham.

TABLE 5

The occurrence of Phacelia distans and of Delphinium parishii with various shrubs, near Palmdale (S. W. Mojave Desert)

The reliability of a first analysis was checked in an adjoining region, where only the occurrence of *Delphinium* was noted, which was percentually almost identical with the first analysis. Mean of first and second analysis used for calculation of percentual occurrence.

	Total No. of shrubs observed in first analysis	*Phacelia distans* Benth. present		*Delphinium parishii* Gray present in		
		No.	Per cent	First analysis	Second analysis	Per cent
Lycium andersonii Gray	18	12	67	3	1	11
Hymenoclea salsola T. & G. . .	8	4	50	1	1	12
Eurotia lanata (Pursh.) Moq. .	17	8	47	0	0	0
Tetradymia spinosa H. & A.	30	12	40	2	2	7
Grayia spinosa (Hook.) Moq.	29	8	28	1	2	5
Yucca brevifolia Engelm.	7	2	28	0	0	0
Ephedra nevadensis Wats.	24	5	21	8	9	36
Stipa speciosa Trin. & Rupr.	35	3	9	5	12	24
Acamptopappus sphaerocephalus (Harv. & Gray) Gray var. *hirtellus* Blake	75	2	3	13	14	18
Eriogonum sp.	18	0	0	4	1	14
Mirabilis bigelovii Gray	6	0	0	1	0	8
Opuntia echinocarpa Engelm. & Bigel.	0	.....	.....	....	1	.....
Dead shrubs	54	3	6	11	12	20
Free growing	....	0	.....	0	1	.....
Total number	321	59	.....	49	56	.....

in connection with the place where it was growing. It will be seen that there is a very close correlation between the frequency of occurrence with a given shrub (see table 1) and the size of *Rafinesquia*. This is an independent indication that the frequency of occurrence of this plant is due to better growing conditions.

If we ask now what conditions are responsible for the occurrence of certain annuals with certain shrubs, the following points should be considered.

(1) The shrub may offer more favorable microclimatic conditions than are found in the open. If this were the case, then we would expect that all shrubs of a given size and foliage density would be equally suitable for given annuals. This is definitely not the case, and shrubs of comparable size and density, such as *Franseria* and *Encelia*, are very different in their aptitude for harboring annuals.

(2) The same thing can be said of the effect of shrubs on the accumulation of seeds by wind, etc. There is generally a preference on the part of the annuals for one side of the shrub, where they will be found growing in greater abundance. This is especially marked with the larger shrubs, and Dr. J. van Overbeek attributes this to the prevailing winds which cause an accumulation of organic material, such as fallen leaves, at the leeward side. This effect of wind, however, does not influence in any way the occurrence of certain annuals with particular shrubs.

(3) One finds many small clusters of shrub-associated annuals growing apart from shrubs, but almost invariably one will find in such clusters the remnants of a shrub in the center of such a cluster. In many clusters it is still possible to identify the dead shrub; and it is most interesting to note how, for instance, the soil around *Encelia* becomes a much better growing medium for *Phacelia* or *Rafinesquia* once the shrub is dead. This observation invalidates the argument that the physical influences of the shrub, such as shading, condition the occurrence of the annuals. That shading is not an important physical factor for the occurrence of many shrub-associated annuals is also evidenced by the fact that cacti, such as *Opuntia echinocarpa* Engelm. & Bigel., offer excellent growing conditions for *Rafinesquia*, e.g.

(4) In some localities the correlations between the shrubs and plants are much more pronounced than in others. This was noted, for example, for *Rafinesquia;* whereas near Barstow not a single specimen was found apart from *Franseria,* in Borego Valley it was, although closely associated, not exclusively found with *Franseria;* and near the station "Ocotillo" most of the *Rafinesquia* grew outside *Franseria* bushes. This was obviously caused by recent floods. Near Barstow the surface of the desert had been undisturbed for many years, and very few plants of any sort grew in the open away from shrubs. Near "Ocotillo" opposite conditions existed. Floods had recently disturbed the surface, and organic material was distributed all over the sur-

face of the desert. In this locality, not only *Rafinesquia,* but a number of other plants commonly associated with shrubs were growing free. This is the same phenomenon that is observed along roads where scrapers have piled up a ridge of scraped-off sand still containing organic material, which is thoroughly mixed. On those ridges, not only shrub-associated annuals grow, but also certain fungi, such as *Podaxon.*

Vegetation in Borego Valley. With the help of Dr. C. Epling, Dr. J. van Overbeek, and Mr. P. Gorham, a sociological study was made of the vegetation of the bajada at the mouth of Palm Canyon. This is a gently sloping field of sand mixed with gravel and occasional large stones. Being an alluvial fan, it is of very recent origin, but the surface apparently had not been disturbed for several years. To get an idea of the general distribution of the flora in this area, all the plants in a square of 8×8 meters were recorded. Within the chosen square which was typical of the region occurred one large shrub, *Hyptis emoryi* Torr., with a crown of 2 meters in diameter, 14 well-developed specimens of *Encelia farinosa* Gray, each with a diameter of approximately 1 meter, and 1 young specimen of the same species, 8 well-developed *Hymenoclea salsola* T. & G. and 2 young specimens. *Nemacladus ramosissimus* Nutt. covered almost half the surface of the bare soil between these shrubs, but because of its thread-like stems and "protective coloration" only close observation revealed its presence. Among the larger annuals *Phacelia distans* and *Langloisia schottii* (Torr.) Greene were the most common, covering about 15 per cent of the surface of the area. The *Phacelia* was growing exclusively with *Encelia* and *Hyptis; Langloisia* was scattered in between the shrubs. Next in abundance were *Chaenactis fremontii* Gray, *Filago arizonica* Gray, and *Bromus rubens* L., covering from 2 to 5 per cent of the surface of the square. Less abundant were *Malacothrix californica* DC., *Euphorbia polycarpa* Benth. var. *hirtella* Boiss., *Mimulus bigelovii* Gray, *Nama demissum* Gray, *Oenothera leptocarpa* Greene, *O. micrantha* Hornem. var. *exfoliata* (Nels.) Munz, *Emmenanthe penduliflora* Benth., *Salvia columbariae* Benth., and *Lotus tomentellus* Greene, each represented by 10 to 40 specimens. Still fewer plants occurred of *Rafinesquia neomexicana, Calyptridium monandrum* Nutt., *Monoptilon bellioides* (Gray) Hall, and *Tillaea erecta* H. & A. Over a larger area, of course, more species were encountered within this region, and table 1, column 1, summarizes the total number of shrubs counted within a transect. This gives a good idea of the frequency of these shrubs on the part of the bajada studied. In addition to the annuals enumerated above, about 15 others were found in this area.

As mentioned above, a number of plants grow predominantly in the open spaces between the shrubs. To these belong: *Mimulus bigelovii, Filago arizonica, Nama demissum, Nemacladus ramosissimus, N. longiflorus* Gray,

Lotus tomentellus, smaller specimens of *Chaenactis fremontii*, *Euphorbia polycarpa*, *Oenothera micrantha*, *O. brevipes* Gray, *Monoptilon bellioides*, *Achyronychia cooperi* T. & G., *Lepidium lasiocarpum* Nutt., *Tillaea erecta*, *Chorizanthe brevicornu* Torr., *Cryptanthe micrantha* (Torr.) Johnston, *Bromus rubens*, and *Eriogonum reniforme* Torr. & Frem. Some of the other plants which are more commonly found near shrubs have been entered in table 1. Columns 2–7 give the percentage of shrubs harboring these annuals. These percentages show that *Phacelia* is common around practically all shrubs, but that with *Dalea*, *Larrea*, and living *Encelia*, fewer specimens are found. Since the abundance of the various shrubs is not the same, the data have been calculated also in a different way, and in columns 8–13 the percentage of the total number of each annual found is entered in connection with the shrub. A critical examination of this table shows that important differences exist in this region between various shrubs as far as their aptitude for harboring different annuals is concerned. As a first example should be mentioned the difference between living and dead *Encelia*. With both *Phacelia* is fairly abundant, but *Malacothrix*, *Emmenanthe*, and *Rafinesquia* are far more common with dead than with living *Encelia*. Whereas *Franseria* is an equally good habitat for *Phacelia* as is dead *Encelia*, it is far poorer for *Emmenanthe* but equally good for *Rafinesquia*. The last columns show that about half of all specimens of *Rafinesquia*, for instance, occur with *Franseria*; whereas only 6 per cent of all *Emmenanthe* plants are found near this shrub.

Under slightly different soil conditions some differences in the specificity of shrubs for certain annuals may be found, but as a general rule it can be said that the less disturbed the soil surface, the more pronounced are the correlations between annuals and shrubs. In a number of other localities less extensive sociological analyses were carried out.

The Mojave desert. In a region north of Kramer the terrain is slightly hilly, with rounded hills of decomposed granite in between the wide washes. Only the hills were investigated by noting the shrub-associated annuals in a transect. Table 3 presents some of the data for this region. The behavior of two *Caulanthus* species, namely, *C. lasiophyllus* and *C. cooperi*, is extremely interesting. The latter species grows quite consistently within shrubs of *Franseria*, although mostly only one single specimen per shrub. It grows occasionally with some of the other shrubs but seems to avoid most of them. Whenever it is growing under *Larrea*, specimens are very small, and only a few times did I see a small specimen growing out in the open. The behavior of *Caulanthus lasiophyllus* is practically complementary to that of the first-named species. It is found only very occasionally with *Franseria*, but is the constant companion of *Larrea*, and also is found more often in the open.

The behavior of *Phacelia tanacetifolia* differs from both of the preceding species in that it is common both under *Franseria* and *Larrea.*

In a different locality just south of the town of Mojave *Phacelia tanacetifolia* behaved differently. It grew there under 70 per cent of all *Larrea* bushes, whereas it was found under only 2 per cent of *Franseria,* which is also quite common there. Only a few plants grew free, but they were extremely short and small, less than one fourth of the size of those growing under the *Larrea.*

Just south of Barstow *Rafinesquia* was found again in limited numbers. It was found there in one transect with 8 of the 50 *Franseria* bushes, whereas under none of the 14 *Larrea,* 3 *Grayia,* and 2 *Mirabilis* was it observed. Near Adelanto *Rafinesquia* was found with 9 per cent of all shrubs of *Atriplex lentiformis* (Torr.) Wats. (?), with 4 per cent of the *Franseria dumosa* Gray, and twice it grew with a dead plant. In not a single place was it found growing free or with *Larrea.* This behavior is similar to that of *Rafinesquia* observed in the Borego desert.

Farther east three localities, two near Ludlow and one south of Amboy, were visited, and the occurrence of *Rafinesquia* was recorded. Table 4 shows the results in a condensed form. Although the abundance of *Rafinesquia* varied greatly, in general the figures agree: Whenever *Krameria* occurs, one has a better than 1 : 3 chance that *Rafinesquia* will be present. For *Franseria* the figures vary more, but in every instance it is a poorer habitat for *Rafinesquia* than *Krameria* is, and twice as good as *Larrea* is. In this connection it should be pointed out that the shrubs of *Larrea* are many times larger than are those of *Franseria,* so that on a purely statistical basis the chance that *Rafinesquia* would occur under *Larrea* is many times greater than under *Franseria.* Since there are also many more other annuals growing under a *Larrea* than under a *Franseria,* there is more chance that one of those others has a favorable influence on *Rafinesquia,* so that the preference of the latter for *Franseria* is far more pronounced than the mere figures in the table indicate.

Another very clear correlation between annuals and shrubs is presented by the occurrence of *Delphinium parishii* Gray in the Mojave desert. Table 5 condenses the data of the observations near Palmdale. The shrubs have been arranged in the order of decreasing occurrence of *Phacelia distans.* For *Delphinium* especially those shrubs are most suitable which do not favor *Phacelia.* Only once were these two annuals found growing together within the same shrub. By independence this would have been expected about 9 times. This proves again, that although the presence of shrubs as such is essential for the occurrence of a number of desert annuals, there is also a strong specificity on the part of certain annuals for certain shrubs.

In a different locality—the hills south of Kramer—*Delphinium* was also quite common, but there half the specimens (70) grew free, in between shrubs. Almost invariably these specimens were smaller than those growing with shrubs. The latter grew predominantly with *Acamptopappus sphaerocephalus* (Harv. & Gray) Gray (47 specimens, with 12 per cent of all shrubs), a few with *Franseria dumosa* (2 per cent), *Aster abatus* Blake (3 per cent), *Eurotia lanata* (Pursh.) Moq. (1 per cent), and 10 plants had developed with dead shrubs.

To get a complete picture of the distribution of plants over the surface of a given desert area, the germination and later growth of the shrubs must also be considered. I have only casual observations to mention. Many of the desert shrubs germinate and develop into young plants only once every few years, when the climatic conditions have been favorable. Even in those favorable years, the number of seedlings is limited. But it is found that *Franseria* seedlings are most often found in open spaces, not associated with pre-existing vegetation. The same seems to be true for *Larrea*. On the other hand, I saw seedlings of *Encelia* growing under other shrubs, such as *Franseria*, so that the common association of *Encelia* and *Franseria* bushes seems to be conditioned by the occurrence of the latter.

On an old lava flow near Amboy seedlings of both annuals and perennials were observed. Sand had filled cracks and depressions in the surface. This sand holds a certain amount of moisture during winter, enough for a few months' growth but not enough to carry any plants through the summer. These lava flows were covered with numerous plants of *Geraea canescens* T. & G., which can grow on any well aerated soil which has not previously had a covering of vegetation. Between the *Geraea* a number of seedlings of *Larrea tridentata* Cov., *Franseria dumosa*, *Atriplex hymenelytra* (Torr.) Wats. and a few other *Atriplex* species, and *Suaeda fruticosa* Forsk. (?) were found; all perennials, which are often found free-growing on the desert. Few annuals had established themselves; in addition to *Geraea*, a few specimens of *Nama demissum*, *Achyronychia cooperi*, and *Cryptanthe* sp. were found, but none of the species commonly associated with shrubs. Thus the vegetation of the lava flow confirms the previous deductions: many shrubs can establish themselves in the desert without association with other plants. The same is true for annuals commonly found in open spaces between shrubs. But the annuals which are normally found only near shrubs were completely lacking on this lava flow; their association with shrubs is essential for their germination and growth.

Another phytosociologically interesting case is presented by the refuse dumps of the harvester ants (*Pogonomyrmex*). These ants collect the fruits of many plants, mostly annuals, which they pick when almost ripe, and trans-

port to their nests. There the fruits are opened, the seeds extracted and stored, and the carpels are thrown outside the nest in semi- or complete circles outside the ring of excavated sand around the nest opening. These refuse heaps are interesting for their flora, and can be recognized one or more years after the nest is abandoned. In such cases a circle of plants will still surround an open space, although it was never occupied by a shrub. The species of annuals occurring in these refuse dumps are different from those of other groups of annuals. In general *Erodium cicutarium* (L.) L'Her. dominates. Even when no other *Erodium* is found in the neighborhood, one may still find a few specimens near the ants' nest. Many plants, which are otherwise found only near shrubs, grow in the refuse dumps, such as *Salvia columbariae, Ellisia micrantha* (Torr.) Brand, *Stephanomeria exigua* Nutt., and around a few nests even *Phacelia distans* was present. One will find the ants carrying the fruits of these plants, so that not only their distribution, but also the accumulation of humus, which is a prerequisite for their growth, is due to the ants. Certain other plants, although growing free on open spaces, are much more frequent near ant nests, such as *Plantago insularis* Eastw. var. *scariosa* Jepson, *Euphorbia polycarpa, Eriogonum* species, and *Langloisia schottii*. This is not due to accumulation of humus since these plants do not grow more abundantly near shrubs. Therefore this must be due to the seed collecting by the ants, and to the occasional oversight of a filled seed pod by the workers. The association of these plants with ant nests is comparable with the epiphytic ant gardens of the tropics (Ule 1904, Docters van Leeuwen 1929), although several of the epiphytes are found almost exclusively in ant nests.

DISCUSSION

By stressing the relationship between certain plants in the desert it is not intended to imply that this is the only or the most important factor in the occurrence of desert annuals. It has been mentioned that many plants are apparently completely independent of other plants and grow most luxuriantly where no shrubs or perennials are established. Nor does this note give all interesting relationships which occur. Almost every trip to the desert brings evidence of other correlations. The frequent occurrence of many annuals with desert shrubs has been previously observed, as evidenced by the habitat description of *Rafinesquia neomexicana*, for example, "Desert Mesas, common among bushes" (Jepson 1925); "Weak-stemmed plant, growing in the shade of shrubs and frequently climbing up through them" (Jaeger 1940). Other quotations from Jaeger run as follows:

Phacelia distans: "common under cat's claw and ironwood."

Phacelia tanacetifolia: "often growing about the bases of creosote or cat's claw bushes."

Caulanthus cooperi: "often growing in the protection of shrubs."

Chaenactis fremontii: "frequent about the bases of creosote bushes." These descriptions show that in addition to the correlation of these annuals with shrubs, Jaeger had even noticed that certain bushes were preferred by certain annuals, but to most of the local taxonomists the specificity of the occurrence of these annuals seems to have been unknown.

If we attempt to summarize, the following conclusions are the logical outcome of the above observations:

In the first place, detritus from most shrubs provides a necessary factor for the growth of all shrub-associated annuals, both for the less specific species, such as *Chaenactis fremontii,* as well as for the specific *Rafinesquia.* For whereas some shrubs in general do not favor the growth of an annual, after their death they become a much better medium (see, e.g., *Malacothrix, Emmenanthe,* and *Rafinesquia* in table 1; their occurrence is increased more than fivefold with dead *Encelia* as compared with living *Encelia*).

In the second place, there is a specific effect of shrubs, which must be ascribed to specific materials given off by them. These materials seem to be produced especially by the living shrub and they determine the specific occurrence of definite annuals. No guess as to the nature of these materials will be given; this can safely be postponed until experimental evidence is available.

In the description of the occurrence of desert annuals it has been pointed out above, that certain physical factors cannot explain the specific occurrence of shrub-associated annuals. For example, shade is not the determining factor, since dead shrubs, with practically all branches gone, provide sometimes a more favorable habitat than living shrubs. If the shrubs acted by virtue of their influence on the water content or water holding capacity of the soil, all shrubs could be lined up in a series of increasing suitability as habitat for annuals, and this series should be the same for different annuals. This is definitely not the case. Besides, by simple reasoning one would expect the shrub to be in water competition with the annuals growing within its confines, and thus inhibit rather than enhance its growth. However, this problem cannot be settled by reasoning, but needs an experimental approach. The preceding observations only serve to provide a factual basis for future experiments, which should be carried out both in natural surroundings and in the laboratory.

It seems likely that the observed phenomenon is widespread and very common in plant communities. In all sociological studies of vegetation made by the author, such a relation between various members of a community was found, and it seems only necessary to point out the phenomenon, to find it in many other instances. Almost every plant-sociological analysis contains

numerous indications that a causal relationship exists between the occurrence of the various members of a plant association; an analysis of the topographical relationships between these plants within the association would probably furnish very strong additional evidence. As soon as a number of such cases have been analyzed the proof of the causality in their mutual occurrence can be given by cultural experiments.

This short note is not the place to discuss more fully the implication of the existence of causal relationships between individual plants. Nevertheless, it should be pointed out that the advantages of rotation of crops might be attributable to such mutual influences. Although it is not usually feasible to mix crops, it is common practice to let one follow the other (crop rotation), and some chemical influences of one crop might be carried over in the soil to the next crop. In many instances a distinct advantage of certain cover crops or shade trees (often leguminous plants) on the main crop has been noted, a beneficial effect which exceeds the effect attributable to nitrogen fixation by the leguminous cover crops. The opposite is also known; the presence of *Imperata*, a common grass in Java, may endanger not only the production but even the life of plantation trees such as *Hevea*.

In lakes, ponds, and ditches distinct correlations between various plants and animals exist. In those localities chemical inter-relations obviously play a major part. It is most interesting to note that lakes in different regions under different climatic conditions may contain the same algal communities. Beyerinck (1927) points out that the same 2 algal biocoenoses which are common in the pools of the heather vegetation in the northern parts of the Netherlands are found in essentially the same floristic composition in the ponds in the southern part of the Netherlands. It seems that only a direct influence of the partners of such an algal community on each other can explain their sociological distinctness.

SUMMARY

Wherever lack of precipitation prevents a continuous cover of vegetation in Southern California, the dependence of certain annuals upon the presence of specific shrubs becomes evident. Especially for *Rafinesquia neomexicana* it was shown that its occurrence depended upon the presence of *Krameria* and *Franseria* in the first place, *Ephedra, Opuntia,* and *Hymenoclea* in the second place, and *Larrea* and *Encelia* in the third place. The aptitude of these shrubs for providing good growing conditions was also evidenced by the fact that the more frequently *Rafinesquia* occurs with a given shrub, the larger are the specimens growing in that shrub. Similar relationships were found for *Caulanthus lasiophyllus, Caulanthus cooperi, Phacelia distans, Phacelia tanacetifolia, Malacothrix californica, Delphinium parishii,* and a

few other annuals. Other groups of annuals do not depend upon shrubs or organic material for their growth. Many of these are found in great abundance in open spaces in between shrubs.

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SECONDARY VASCULAR TISSUES OF THE OAKS INDIGENOUS TO THE UNITED STATES—III. A COMPARATIVE ANATOMICAL STUDY OF THE WOOD OF LEUCOBALANUS AND ERYTHROBALANUS[1]

SIMON WILLIAMS[2]

(WITH FIFTEEN FIGURES)

Leucobalanus and *Erythrobalanus* have been redefined previously on the basis of the correlation existing between leaf-form and the structure of the late-wood pores in the secondary xylem (18). The nature and distribution of tyloses among *Quercus* species has been discussed in relation to these newly proposed subgeneric limits (19). The controversial comments stimulated by these initial reports activated a more comprehensive anatomical study of the two subgenera to determine in what other respects the red and white oaks, as recognized herein, differ from one another. To this end, the various cell and tissue types present in oak wood are discussed separately below.[3]

CELL TYPES AND TISSUES STUDIED

Early-wood Vessels. The white oaks are typically ring-porous with the exception of the shrubby species, *Q. sadleriana* R. Br. Campst. The early-wood zone ranges from 1- to 4-seriate, and in a given species may exhibit this entire range. In general, in the more slowly growing species, the larger pores are more nearly arranged in a uniseriate row, and those further out in the early wood are considerably reduced both in number and size. The red oaks are similar in these respects, with the exception that the woods may range from typically ring-porous to diffuse-porous. Also, the pores of the red oaks exhibit a less abrupt transition in size from early wood to late wood.

The shape of the pores is similar in both groups, ranging from nearly orbicular to oval or narrowly elliptical. The radial axis is generally longer than the tangential, although it is not so conservative in its variation as the latter.

In typically ring-porous species of the two subgenera, the wall of the early-wood pores ranges between 2 and 4 microns in thickness. Among the

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1 The information incorporated in this paper has resulted from research by the author while a member of the Department of Wood Technology, New York State College of Forestry, Syracuse, N. Y.

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3 One hundred and thirty-five samples, covering thirteen species of *Leucobalanus* and thirty-two species of *Erythrobalanus*, were included in this study.

diffuse-porous species, the early-wood pores are somewhat thicker walled (3–6 microns) and grade more gradually into the thick-walled late-wood pores (all the diffuse-porous species are red oaks).

No distinction between the two subgenera can be based either on the length or the shape of the vessel-members. The early-wood vessel-members are usually short, but occasionally may be classified as long; the xerophytic and shrubby species have shorter elements. The early-wood vessel-members are from 50 to 125 microns shorter, on the average, than those in the late wood; they may be tailed or not; if present, the tail is usually short and is either pointed or tapers to a blunt apex.[4] The perforation plates are simple and horizontal or nearly so. Three types of pitting are common throughout the vessel-members of the early wood of all species, viz.:[5]

1. Pits leading to vasicentric tracheids (fig. 1). This type of pitting is the most frequent and may extend entirely over both the radial and tangential walls. Usually, the vermiform impressions of the tracheids are present; when they are, the pitting is restricted to these areas. The pits are large and round, and are provided with an elliptical to oval, included aperture. They vary in diameter from 4 to 12 microns. Not infrequently they appear punctate in surface view, resembling vestured pits in every way. However, this feature is sporadic in *Quercus*, and as has been pointed out (2), it is the result of artifacts produced during post-mortem changes in the cells or during the transformation of sapwood into heartwood. No structural evidence could be found to contradict Bailey's statement on this matter.

2. Ray-crossing pits (fig. 1). Ray-crossing pits are restricted to pit fields. The number and size of the pits within a pit field varies according to the size of the field from 3 to 7 or more. The pits are usually simple, but may be bordered. They are extremely variable in shape, ranging through round, oval, twisted-elliptical, rectangular, etc., and in alignment they exhibit fully as wide a fluctuation. The long axis may be as much as 30 or more microns; in general, the larger the pore, the larger the pit field, and the more conspicuous the pits. This is not absolute for the oaks.

3. Pits leading to longitudinal parenchyma. These are rounded, bordered pits with a broad, oval, included aperture; they are noticeably smaller (from 3 to 6 microns in diameter) than the intertracheary pits. Pits of this type may be recognized by their tier-like arrangement in pit clusters, as seen in macerated material.

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[4] Vessel members measured and described after the methods suggested by Chalk and Chattaway (5, 7).

[5] The vessels are always solitary in *Quercus;* if intervessel pitting occurs, it is restricted to the overlapping tails of members of the same vessel, and resembles in every way the pitting between a vessel and a contiguous vasicentric tracheid.

Late-wood Vessels. The structure and distributional frequency of the late-wood pores provide the only absolute means, on the basis of wood anatomy, of distinguishing *Leucobalanus* from *Erythrobalanus* (18). Abromeit (1), in 1884, first attempted to classify *Quercus* species on the basis of wood anatomy, depending largely upon variations in frequency and cell wall thickness of the late-wood pores to delimit his various groups. Unfortunately, probably through insufficient sampling and coarseness of technique, Abromeit made several errors, such as classifying *Q. wislizenii* A. DC. with those oaks having thin-walled late-wood pores and utilizing extremely variable characters such as pore frequency, pore size (vaguely defined as small, large, etc.), and the degree of lateral coalescence of the radially aligned late-wood pore groups. Because of these various factors, the subdivisions created by Abromeit could not be recreated by examination of new material, nor do these subdivisions adhere strictly to the subgeneric limits defined either by Williams or other workers in this field. Further, Abromeit did not include in his study any of the eight controversial species which Williams has shifted from *Leucobalanus* to *Erythrobalanus*. The work of Abromeit, while of historical significance, cannot be considered as bearing directly upon the new classification of *Quercus* species brought out in this current series of reports on the wood anatomy of oak species native to the United States.

A summation of the differences which are of diagnostic significance follows:

The late-wood pores of white oaks are characterized as follows:

A. *At low magnifications (up to 10×)*

1. Minute, in the majority of species barely distinguishable as distinct openings; when visible distinctly angled and thin-walled; extremely numerous and so crowded in radially aligned, flame-shaped patches that they cannot be counted.

B. *At high magnifications (50× or more)*

1. Thin-walled (under 3 microns)
2. Distinctly angled (more or less polygonal)
3. Very numerous (ranging from 30 to 125 or more per mm.^{2}, for average growth rates generally 40 to 70 per mm.^{2})

The late-wood pores of red oaks are characterized as follows:

A. *At low magnifications (up to 10×)*

1. Usually distinct with a hand lens, appearing as rounded, thick-walled orifices, which are quite widely spaced (and hence easily counted) and more or less radially aligned.

B. *At high magnifications (50× or more)*

1. Thick-walled (3 to 10, average 4 to 7 microns).
2. Rounded or oval (the outer wall obscurely angled).
3. Not numerous (relatively), ranging from 8 to 25 per mm.^{2}, for average growth rates generally 10 to 16 per mm.^{2} (this figure did not exceed 30 in any sample studied, in spite of the fact that every effort was made to obtain the largest figure possible in certain test cases).

The late-wood vessel-members range from short to long. Although *Leucobalanus* and *Erythrobalanus* cannot be separated on this basis, the white oaks tend to have slightly longer members, with longer, more tapering tails than those of the red oaks. In the latter, the vessel-members taper more abruptly to an acute or acuminate apex. In both groups, the end walls are oblique and the perforation plates simple, except that scalariform plates may occur in the smaller vessels of the live oaks. This feature was not observed during the present investigation, but has been reported from different sources (1, 14, 17). The pitting on the walls of the late-wood members is similar in every way to that found on the early-wood members.

Fibers. The fibers of the oaks exhibit no departures of subgeneric significance (fig. 2). They are intermediate between typical fiber-tracheids and libriform fibers found in other woods, and except for length are remarkably uniform throughout the genus. Thick-walled fibers are the rule, although thin- and very thick-walled fibers occur occasionally.

The shape of the fibers is best determined from macerated material; they are elongated, tapering elements which frequently possess forked or jagged-toothed edges. In cross-section, the fibers are generally hexagonal, but owing to pressure occasioned by the enlargement of the vessels behind the cambium, they depart from this shape considerably. The tangential diameter varies from 6 to 25 (average 10–16) microns, and the walls vary in thickness from 4 to 10 microns. Not infrequently the inner wall (next to the lumen) is gelatinous, but this feature is common to both subgenera and hence is of no diagnostic importance. Large masses of gelatinous fibers occur in some oaks (*Q. lyrata* Walt.) as a constant feature; in others, fibers of this sort are abundant but not massed or may be wanting entirely. The gelatinous wall, when present, is usually thick, constituting from one-third to one-half or more of the cell wall.

The interfiber pits, although bordered, are so small that the classification of the fibers on this basis is of no value except at extremely high magnifications. They are round, possess slit-like excluded apertures, and are approximately 4 microns in diameter. Interfiber pitting is rare on the tangential walls and sparse on the radial walls (occasionally moderately abundant over a small area).

Gummy infiltration is common in the fiber lumina. It often assumes the form of spindle-shaped or rod-like bars across the cavity which simulate the cross-walls of septate fibers.

Several statements relative to the variation in the fibers of oak wood may be made here, even though they are of no significance diagnostically. Although in any one species both long and short fibers occur in different samples and in the same sample, the shrubby and xerophytic oaks, as groups,

have shorter and finer fibers than the remaining species; this observation agrees with the work of Bailey and others (3, 15, 16). This phenomenon has probably resulted from the adaptation of these species to edaphic and climatic factors, and although it was undoubtedly conditioned by the environment, it has now become fixed, that is, it has now become a specific feature for the oaks in question. Mell (11), working with black walnut (*Juglans nigra* L.), has pointed out that the faster-growing trees of this species on better sites have longer elements (fibers) than those growing on poorer sites, with resulting slower growth rates. There is no indication that this holds for the oaks; except under very unusual conditions (xeric habitat), no variation in fiber length can be anticipated in individuals of the same species grown under optimum and unfavorable conditions. Nor does suppression in oaks have any observable effect on fiber length, contrary to the results of MacMillan (10) working with red spruce (*Picea rubra* Diet.). MacMillan indicates that suppressed trees of this species have shorter elements than free-grown trees. Although the field data covering the samples of oak studied were meagre for some species, there is no indication that oaks react similarly to red spruce. Suppression usually implies slow growth (although slow growth does not necessarily imply suppression), and fiber length in oak appears independent of ring width.

Longitudinal Parenchyma (figs. 4, 5, 6). The arrangement of longitudinal parenchyma is similar in both subgenera; in the great majority of species it is metatracheal, with occasional cells terminal or contiguous to the vessels (paratracheal). The latter type, after the reasoning of Chalk (6), is probably incidental owing to the irregular course of the bands of metatracheal parenchyma.

The factors causing variation in the frequency distribution of parenchyma are so complex that they cannot be evaluated with any real accuracy. Frequency would, therefore, be too uncertain a feature to be used in defining subgeneric limits, or in aiding in the recognition of any congeries within subgenera. Nevertheless, there does seem to be a vague indication of group relationships, based on frequency, that is worthy of note. *Erythrobalanus*, especially the live oak group, appears to have a greater amount of parenchyma, on the average, than *Leucobalanus*. The species studied are listed below, with the average obtained for the number of parenchyma cells per mm.^{2} of late wood, exclusive of the area occupied by the pores.[6]

The bands of zonate parenchyma are commonly 1- or 2-seriate in the white oaks, and seldom anastomose. The increased parenchyma volume of the red oaks is manifested by wider bands (2 to 4 or more seriate), and the

[6] These averages are not calculated statistically, but each is the average of ten measurements from each sample of every species.

Number of Cells per Square Millimeter of Longitudinal Parenchyma

Leucobalanus

Species	*no. mm.²*
Q. alba	325
Q. bicolor	300
Q. durandii	560
Q. garryana	400
*Q. breweri**	675
Q. lobata	490
Q. lyrata	
Q. macrocarpa	390
Q. muhlenbergii	360
Q. prinus	305
*Q. sadleriana**	310
Q. utahensis	390

Erythrobalanus

Live Oaks

Species	*no. mm.²*
Q. agrifolia	640
Q. arizonica	620
Q. chrysolepis	765
*Q. dumosa**	690
Q. engelmannii	475
Q. emoryi	540
Q. laurifolia	530
Q. myrtifolia	595
Q. oblongifolia	435
Q. hypoleuca	650
*Q. reticulata**	545
Q. virginiana	610
Q. virginiana geminata	620
Q. wislizenii	475

Deciduous Oaks

Q. borealis maxima	375
Q. catesbaei	530
Q. cinerea	595
Q. coccinea	355
Q. douglasii	855
Q. ellipsoidalis	460
Q. ilicifolia	555
Q. imbricaria	390
Q. kelloggii	600
Q. marilandica	600
*Q. morehus**	575
Q. nigra	410
Q. palustris	370
Q. phellos	435
Q. falcata	490
Q. falcata triloba	510
Q. falcata leucophylla	410
Q. shumardii	420
*Q. vacciniifolia**	615
Q. velutina	410

*Shrubby species.

frequent coalescence of these. Among the live oaks, in which parenchyma is unusually abundant, the zonate arrangement is obscured.

In all other respects, the parenchyma of both groups is identical. In cross-section, the cells are thin-walled (under 4 microns) and range from

oval to polygonal due to tissue distortion occasioned by the enlargement of the vessels behind the cambium. In this plane, they are distinctly larger than the fibers, measuring from 10 to 30 or more microns in tangential diameter. In longitudinal section, the strands of parenchyma are composed of from 2 to 20 or more units, the terminal cells tapering to a point. The pits between cells are simple and abundant, and when seen in face view show a tendency to cluster into small groups of from 2 to 4 in pit fields. Crystals may be absent, sparse, or very abundant.

Tyloses. The nature of the tyloses in the two subgenera of *Quercus* has been previously discussed and needs no further consideration in this paper (19).

Broad Rays. The broad rays of *Quercus* are either aggregate or compound. *Leucobalanus*, with the exception of the shrubby forms, has true compound rays (figs. 10, 11). Both true compound rays and aggregate rays characterize *Erythrobalanus*, but only among the live oaks do aggregate rays become a constant feature of the mature wood (figs. 12–15). In this regard, the live oak group is more strikingly distinguished by the nature of the broad rays than by any other structural feature with the possible exception of their diffuse-porousness. The tendency to possess aggregate rays, taken together with the exceptional width, shortness along the grain, blunt ends, and high frequency of these structures, sets these species off as distinct from other oaks. This composite of features was noted by Sudworth and Mell (17), who state "Evergreen oaks, and particularly very slow growing species or those growing on dry soil, develop low and wide rays, which have blunt ends above and below."

As with longitudinal parenchyma, the size and frequency distribution of the broad rays seem to indicate certain group relationships. However, here too the inherent range of variation, either by species or by groups, is greatly complicated by the influence of the environment; until comprehensive studies can be made on each individual species throughout its entire range, these factors cannot be truly evaluated, and the significance of differences must remain in doubt. There is, of course, no reason to assume that the oaks do not follow the general laws of ray size and ray distribution (4, 8, 12), which may be listed as follows:

1. Ray volume is at a maximum in the root and in the crown; in the former because of increased size of the rays, in the latter because of an increase in their number.
2. Ray volume increases with age up to a certain point, after which it remains more or less stationary.
3. The larger the crown and the greater the physiological activity, the greater the ray volume.

4. Ray height decreases from the stump upward, and generally increases from the pith outward (Büsgen and Münch 4, state that in some conifers the ray height is greatest in the first ring, decreasing thereafter for a period, and again increasing with old age).

Granting that these rules are in force among the oaks, there is still no indication of the effect of external factors in regulating the quality and quantity of the changes within a species or within an individual of a species. The rules may be valid, but they offer no clue in determining the specific nature of apparent differences in size and distribution. The only hint as to the reaction of the oaks to changes in site and locality lies in the work of Myer (13) on *Q. alba* L. This author, after studying the effect of range, habitat, and position in the tree on the structure and strength of this species, concludes, in relation to the broad rays:

"The height of the compound ray was found to be about 50% greater in specimens from the Ohio Valley and Appalachians than it was in those specimens from northeastern United States. Little weight can be attributed to this difference, however, since it must be contrasted to a 90% average intra-regional variation. Within a single tree the maximum deviation in ray height was found to be as high as 60% in passing from stump to merchantable top. The highest rays were found in the stump wood and from this point their height decreased gradually upward. Radially in the trunk, the rays became slightly taller as they progressed from the pith outward but the percentage change of 30% was less than it was lengthwise in the bole. Although these figures show where the highest rays are likely to occur, it nevertheless is quite evident that range exerts no constant influence on this dimension which appears to be more dependent upon individual differences and upon the region in the range where the tree is growing."

If the variation exhibited by *Q. alba* is indicative of specific instability, then the range between different species is liable to be even greater and less applicable in diagnosis. Therefore, no attempt was made to utilize the differences exhibited between species in the character of the broad rays, aside from certain broad generalizations, as follows:

1. As stated previously, the evergreen habit favors short, broad rays (17), which exhibit a distinct tendency toward the aggregate condition.

2. Dry site, in both subgenera, results in a wider type of ray, but has no constant influence on ray height.

3. The effect of abundant moisture is exceedingly contradictory. Sudworth and Mell state "the deciduous oaks, growing in moist alluvial soils, develop high and narrow pith rays." In general, this is true, but increased moisture may influence ray width in both directions. *Q. bicolor* Willd., for example, has broad high rays, in contrast to the narrow rays developed in *Q. phellos* L. and *Q. lyrata* Walt.

4. As a group the red oaks have shorter, more slender rays, and a higher ray volume than the white oaks (referring to the broad rays). This is in general agreement with the work of Myer (12) and Hartig and Eichler (Büsgen and Münch, p. 116), and although no ray volume measurements were made, the statement is given further substantiation by the ray frequency counts that were made. For *Leucobalanus,* the average range for the number of rays per square inch of tangential surface was 20 to 34; the entire range 10 to 60. Among the red oaks, the average range was 30 to 65; the entire range 25 to 115. There was considerable overlapping between the two subgenera, as is indicated by the breadth of the spread through which they both passed. The live oaks were all high (with the exception of those samples of *Q. chrysolepis* Liebm., in which the state of aggregation is so weak that the broad rays are inconspicuous), ranging from 50 up.

Measurements revealed that the average ray height among the red oaks rarely exceeds one-half inch, further that the maximum ray height of the samples studied was 2 inches. Rays over one-and-one-half inches in height are not common. In *Leucobalanus,* while many of the samples fell into the same general range as those of *Erythrobalanus,* an equal number of specimens, especially from such species as *Q. alba* L., *Q. bicolor* Willd., *Q. stellata* Wang., and *Q. montana* Willd., had average ray heights of from one-half to one-and-one-quarter inches, and rays in excess of one-and-one-half inches were common. The maximum observed height was 4 inches. The live oaks had the shortest rays of all, on the average, ranging from one-eighth to three-eighths of an inch, rarely exceeding one-half inch. Longer rays do occur among certain species of live oak, such as *Q. arizonica* Sarg., *Q. dumosa* Nutt., and *Q. virginiana geminata* Small. Frequently, in this group, the influence of ray aggregation extends for a considerable distance along the grain, creating a false impression of high compound rays.

Uniseriate Rays. The uniseriate rays of *Quercus* spp. are valueless for diagnostic purposes, except as they are typical for the genus. They are very abundant, ranging from 75 to 200 or more per mm.[2] (tangential surface); vary from 2 to 30 or more cells and up to 500 microns in height (average 125–300); are largely homogeneous; are frequently biseriate in part in the immediate vicinity of the broad rays; the cells are oval to rectangular (tangential surface), those in the early wood coarse and more rounded (10–20 microns in tangential diameter) than those in the late wood (7–15 microns in tangential diameter); crystals are infrequent; infiltration is sparse.

Crystals. Crystal formation is common on the wood of all *Quercus* species; it is most abundant in the xerophytic live oaks. In general, the better-site, deciduous oaks have the fewest crystals, the drier-site deciduous

oaks have moderately abundant crystals, and the xerophytic live oaks have excessively abundant crystals. This sequence is variable, any species being capable of exhibiting wide extremes from sample to sample. Although the range of material was inadequate for a thorough test, it may be said as a rule that heartwood contains more crystals than sapwood.

The crystals are largely rhomboidal to hexagonal in section, and are restricted to the parenchymatous tissues. They are most abundant in the broad rays, especially in the neighborhood of the ray ends. The loculi may be solitary or in strings of 2 to 30 or more. The type of crystal formation in oaks has been arbitrarily defined in this paper as sparse (fig. 7), moderately abundant (fig. 8), and excessively abundant (fig. 9).

Color. Not only the tyloses, but the color of the wood has long been used as an aid in distinguishing red and white oaks. In general, the heartwood of the red oaks was presumed to have a distinct reddish tinge, while that of the white oaks was considered to be of varying shades of brown, without a carneous cast. However, even before the redefinition of the two subgenera, and the subsequent shift of eight of the so-called white oaks to the red oak group, as suggested in the first paper of this series, color was recognized as not being wholly satisfactory diagnostically. The shifting of these eight species, if it is accepted by taxonomists, renders color even less acceptable than before in determining subgeneric limits. But with this shift, color has assumed a new significance in isolated cases, serving as a means to distinguish species which are otherwise very similar. For example, *Q. arizonica* Sarg. may be separated from *Q. emoryi* Torr. by the color of the sapwood; it has a distinct reddish cast in the latter and ranges from creamy white to tan or light brown in the former, without a reddish tinge.

Vasicentric Tracheids. The shape, length, type of pitting, and general arrangement of the vasicentric tracheids are similar throughout the genus.

Explanation of figures 1–9

FIG. 1. *Q. velutina* Lam. Note the large ray-crossing pits, restricted to definite pit fields, also the vermiform vasicentric tracheids with large bordered pits. ×430. FIG. 2. *Q. alba* L. Note the thick-walled fibers; also gelatinous fibers scattered over the section, the gelatinous layer appearing darker. ×450. FIG. 3. *Q. velutina* Lam. A mass of vasicentric tracheids sheathing a vessel. ×285. FIG. 4. *Q. alba* L, Narrow, fairly regular bands of zonate parenchyma, characteristic of many white oaks and uncommon among the red oaks. ×240. FIG. 5. *Q. imbricaria* Michx. Wider and less even bands of parenchyma than those in figure 4, more common among the eastern deciduous red oaks than elsewhere. ×240. FIG. 6. *Q. virginiana geminata* Sarg. Note the abundance of the parenchyma and that the zonation is obscured. This type of distribution characterized the live oak group. ×240. FIG. 7. *Q. palustris* Muench. Tangential section showing sparse crystal formation. ×180. FIG. 8. *Q. Prinus* L. Tangential section showing moderately abundant crystal formation. ×180. FIG. 9. *Q. Emoryi* Torr. Tangential section showing excessively abundant crystal formation. ×180.

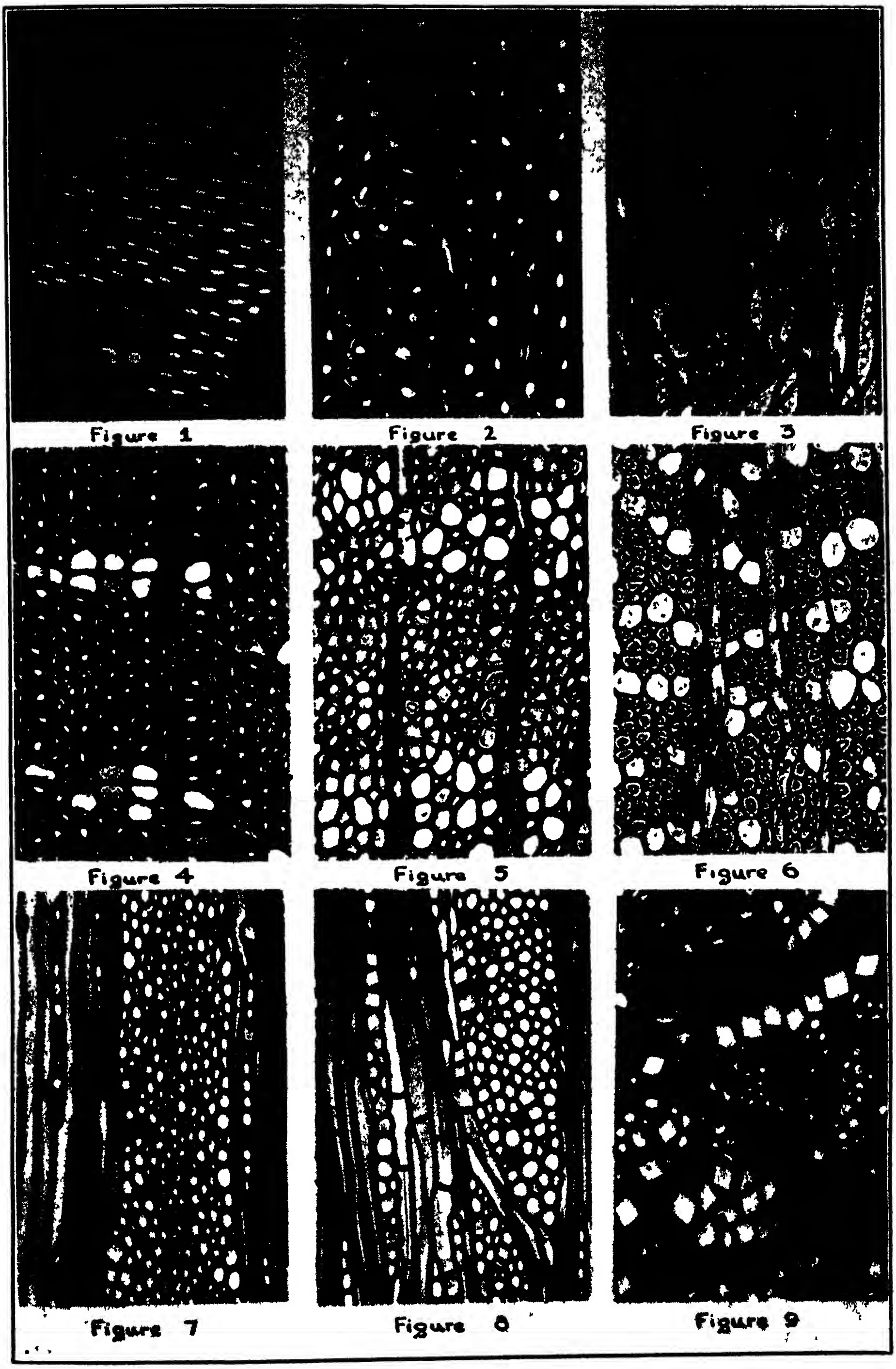


Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6
Figure 7
Figure 8
Figure 9

Units of this type are restricted to the immediate vicinity of the vessels, about which they form multiseriate sheaths consisting of vermiform, profusely pitted (bordered, with an oval, included aperture) cells intermingled with strands of longitudinal parenchyma (fig. 3). The length of the tracheids fluctuates within remarkably small limits throughout the genus, averaging between 550 and 700 microns, or approximately one-half of the average fiber length. As is true of other cells, although much less markedly, the tracheids are smaller in the xerophytic and shrubby oaks than elsewhere. Further, the larger the pore, the shorter and more twisted the tracheids associated with it.

In macerated material, vasicentric tracheids are extremely variable in shape. The majority are slightly twisted, with more or less blunt apices; others are forked at one or both ends; some have horizontal end walls; many are swollen at one end and taper into an elongated, thread-like extension at the other end; a few taper evenly at both ends and resemble fibers in outline. The last type can easily be distinguished from fibers by the abundance of pits and the type of pit-sculpture on the walls. Intertracheid pitting and the tracheid-vessel pitting are similar and have already been described in the discussion of the vessels.

Miscellaneous Data. Except as already indicated, weight, thickness of sapwood, etc., which could not be thoroughly substantiated in this research and are hence of unknown value in distinguishing species, will not be considered further.

SUMMARY AND CONCLUSIONS

In addition to the indications of subgeneric limits presented by the correlation existing between leaf-form and the structure of the late-wood pores and the type and distribution of tyloses, several additional indications of the validity of these limits are noted. While none of these permits of an absolute segregation of species comparable to that described on the basis of leaf-form and late-wood pore anatomy, they are of value and may be enumerated as follows:

1. The frequency of longitudinal parenchyma is noticeably higher in *Erythrobalanus* than in *Leucobalanus*. This is especially true of the live oak group in *Erythrobalanus*, in which parenchyma is often so abundant that the

Explanation of figures 10–15

FIG. 10. *Q. imbricaria* Michx. Broad ray type of deciduous red oaks. Pattern formed on tangential surface of wood. ×2.5. FIG. 11. *Q. imbricaria* Michx. Portion of broad ray highly magnified. The shape of the cells of both uniseriale and broad ray is typical for the genus. ×100. FIG. 12. *Q. virginiana* Mill. Pattern formed by broad rays on tangential surface of wood. ×2.5. FIG. 13. *Q. virginiana* Mill. Broad ray highly magnified. Note aggregate ray condition typical of many live oaks. ×100. FIG. 14. *Q. stellata* Wang. Pattern formed by broad rays on tangential surface of wood. ×2.5. FIG. 15. *Q. stellata* Wang. Broad ray highly magnified. ×100.

Figure 10

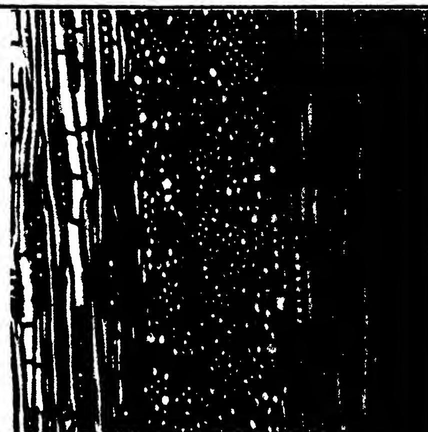
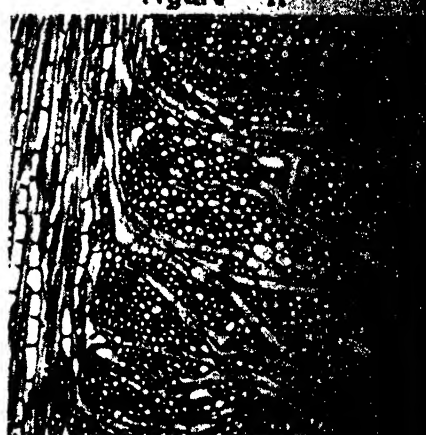


Figure 11

Figure 12



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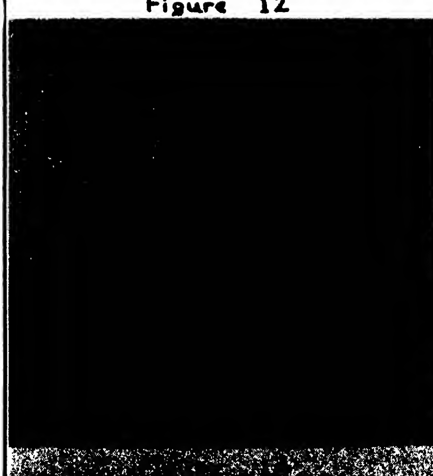

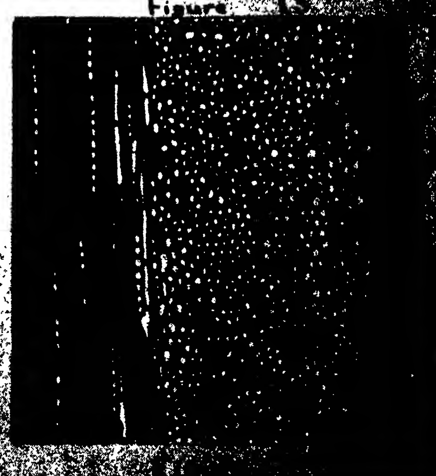

zonate arrangement which typifies the genus *Quercus* is obscured. The shrubby species tend toward a high parenchyma volume, irrespective of the subgenus. The size, shape, type of pitting, etc., of the cells are similar throughout the genus.

2. The broad rays of *Erythrobalanus* are generally shorter and more abundant than those of *Leucobalanus*. The live oaks, in *Erythrobalanus*, are distinct from the deciduous red oaks on the basis of the very short, stubby, extremely broad, excessively abundant rays, many of which are typically aggregate. Among the deciduous red and white oaks, there is considerable overlapping in height and width; however, whereas the average ray height among the red oaks rarely exceeds one-half inch, average ray heights of from three-fourths to one-and-one-half inches are common among the white oaks. In general, dry site stimulates broader rays; dry site plus evergreen habit very broad, but shorter rays with a distinct tendency toward the production of rays of the aggregate type; wet site appears to exert no constant directional influence on width or height.

3. Certain physical distinctions hold between the woods of the red and white oaks. For example, the color of the heartwood may be used with reservation; the white oaks have from tan to dark brown heartwood, devoid of reddish tinge, while the red oaks, in contrast, have heartwood with a roseate cast; there is considerable overlapping in this regard. As regards weight, the literature reveals that the live oaks have the heaviest woods and the deciduous red oaks the lightest. The wood of all of the species studied was odorless and tasteless. No hardness tests were made.

In all other respects, the woods of *Erythrobalanus* and *Leucobalanus* merge insensibly in their structural and dimensional features.

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CULTURE OF ISOLATED ROOTS OF ACACIA MELANOXYLON

JAMES BONNER

INTRODUCTION

The culture of isolated roots of several herbaceous species of plants over extended periods has been previously reported by various authors. The present paper deals with preliminary observations concerning the culture of isolated roots of a tree species, *Acacia melanoxylon*.

METHODS

Seeds were collected in the fall from large trees and were disinfected by a ten minute treatment with 0.1 per cent $HgCl_2$. Boiling water was next poured over the seeds, which were then allowed to soak for 24 hours. Heat treatment of these seeds appeared to be essential to germination. Germination took place on moist sterile filter paper contained in Petri dishes. Root tips approximately 1 cm. long were removed from the seedling roots and transferred to Petri dishes containing 20 cc. of nutrient medium[1] similar to that used in other investigations (1). All of the cultures were maintained in the dark at 25° C.

EXPERIMENTAL RESULTS

In the first experiment, 6 roots were maintained in the basal medium containing supplements of thiamin, pyridoxine (each 0.1 mg. per liter) and nicotinic acid (0.5 mg. per liter). The roots were subcultured at weekly intervals for 20 weeks by removal of 1 cm. tips to fresh medium. During the first week of culture the average growth increment was 12 mm., while during the twentieth week an average of 11 mm. of growth in length took place. That the growth rate remained roughly constant during the 20 weeks is indicated by the average weekly growth increments for successive 5 week periods, which were respectively 9, 9, 8, and 8 mm. per week.

The growth rate of *Acacia* roots as indicated by the above experiment was markedly smaller than that obtained for certain isolated roots of herbaceous plants such as tomato, clover, and alfalfa (1), grown under similar conditions. Various alterations in the nutrient medium did not, however, result in increased growth of *Acacia* roots. These alterations included: use of solid or semi-solid substrata (0.8 and 1.5 per cent agar media) use of 4, 6, or 10 per cent sucrose in place of 2 per cent, addition of yeast extract or whole

[1] This medium contained per liter of redistilled water; 236 mgs. $Ca(NO_3)_2 \cdot 4H_2O$, 36 mgs. $MgSO_4 \cdot 7H_2O$, 81 mgs. KNO_3, 65 mgs. KCl, 20 mgs. KH_2PO_4, 1.5 mgs. ferric tartrate, and 20 gms. sucrose.

dried yeast in various concentrations, and addition of micro-nutrients (i.e., in addition to those already contained in the medium as impurities) including Zn, Cu, Mn, Mo, and B, to the medium. The mean growth per week was likewise not increased by the use of transfer periods of 2, 3, 4, or more weeks rather than the usual weekly period. The growth of isolated *Acacia* roots under the present conditions was however somewhat slower than that of roots grown under similar conditions but attached to seeds (17.5 mm. per week over a 2 week period) or grown as seedlings in Hoagland's solution in the greenhouse (12.5 mm. per week). It would appear that optimal conditions for the culture of isolated *Acacia* roots were not realized.

In a further experiment, the effect of varied supplements was investigated; the results are given in table 1. Roots maintained in medium contain-

TABLE 1

Influence of various substances on the growth of isolated Acacia roots

Supplements to basal medium	Number of roots	Growth in mm. per week					
		week 1	3	5	15	26	35
None	11	5.5	1.8	0.0	0.0	0.0	0.0
B_1 alone	13	5.0	3.2	0.6	0.0	0.0	0.0
Nicotinic acid alone	15	6.0	2.2	1.0	0.0	0.0	0.0
B_6 alone	12	7.3	3.8	0.0	0.0	0.0	0.0
B_1 + nicotinic acid	17	5.4	6.5	6.0	3.1	0.0	0.0
Nicotinic acid + B_6	13	6.0	4.6	1.6	0.7	0.0	0.0
B_1 + B_6	12	5.9	3.9	1.7	1.4	0.0	0.0
B_1 + nicotinic acid + B_6	17	10.6	8.9	8.9	8.2	6.6	10.0

ing no supplement, or in medium containing only one of the three supplementary substances, grew less in each successive transfer and ceased or nearly ceased growing within 5 weeks. The same was true of roots grown in medium containing supplements of nicotinic acid and pyridoxine or thiamin and pyridoxine. Roots which received thiamin, nicotinic acid, and pyridoxine continued to grow for 35 weeks (averaging 8 ± 0.3 mm. per week) at which time they were discarded. Roots which received only thiamin and nicotinic acid grew somewhat more per week and continued growing for more weeks than roots on other deficient media, but ultimately lagged behind roots receiving all three growth factors. This experiment suggests then that thiamin, nicotinic acid, and pyridoxine all exert positive effects on the growth of isolated *Acacia* roots.

The roots grown in the above experiments were distinguished by their lack of branches and by their lack of secondary thickening. It was not found possible to induce the formation of branch roots. The bases of roots (after removal of the tip) were maintained in nutrient solution for from 1 to 4 weeks without the appearance of branches. In one experiment, 6 roots were

transferred intact (without subculturing) to fresh medium at weekly intervals for 16 weeks. At the end of this period the roots had attained an average total length of 81 mm., although the weekly growth increment had decreased to 0.9 mm. No branches appeared and the roots did not exhibit any marked secondary thickening.

OTHER SPECIES

Unsuccessful attempts were made to cultivate roots from other species of woody plants. In every case liquid as well as solid media were used, and various supplements and sucrose concentrations were tried as with *Acacia*. In three species (grape, lemon, grapefruit) the roots failed to grow at all after excision, a state of affairs which has been met with in some herbaceous plants, notably certain of the *Cucurbitaceae*. In 5 species (*Poinciana gilesii*, orange, *Simmondsia california, Thuja orientalis, Parthenium argentatum*), the roots regularly grew for one or more weeks after excision, but ceased growing within three weeks. The average growth in the first week of culture varied from 7 mm. (*Simmondsia*) to 22 mm. (*Poinciana*). In three species a few roots were cultivated through several transfers. Thus, with *Sterculia diversifolia* growth averaged 9 mm. in the first week. Eighty of eighty-two roots did not grow after the fourth transfer. One individual root however was maintained through 21 weekly transfers (in liquid medium supplemented with thiamin, nicotinic acid, and pyridoxine) at an average growth rate of 8 mm. per week, and a second root was maintained through 13 weekly transfers at an average growth rate of 8 mm. per week. Such exceptional roots were also found in the cases of *Bauhinia purpurea* and *Wisteria sinensis* (see also Robbins and Maneval, 2). In all cases, however, the growth rates were low and similar to that of isolated *Acacia* roots rather than to those characteristic of the herbaceous roots previously studied.

SUMMARY

1. Isolated roots of *Acacia melanoxylon* were cultivated for periods up to 35 weeks, in liquid medium containing inorganic salts, 2 per cent sucrose, and supplements of thiamin, pyridoxine, and nicotinic acid. All of these supplements appeared to be essential to the continued growth of isolated *Acacia* roots although the absence of pyridoxine was manifested less strikingly than absence of thiamin or nicotinic acid.

2. The growth rate of isolated *Acacia* roots averaged 8 ± 0.3 mm. per week over a period of 35 weeks. This growth rate, which was about two-thirds that of roots of seedling plants, was in marked contrast to the 40 to 150 or more mm. per week typical of isolated roots of herbaceous species as tomato, alfalfa and clover.

3. No formation of branch roots or extensive secondary thickening was observed in isolated *Acacia* roots.

4. Continued growth in vitro could not be obtained with isolated roots of 12 other species of woody plants with the exception of rare individual roots of *Sterculia diversifolia, Bauhinia purpurea* and *Wisteria sinensis.*

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CYTOGEOGRAPHY OF OXYDENDRUM ARBOREUM[1]

J. T. BALDWIN, JR.

Oxydendrum DC. is an ericaceous monotype: *O. arboreum* (L.) DC. Though usually a small tree, it may attain a height of more than seventy feet and a trunk diameter of twenty inches (Hough 1924). The leaves and stem are somewhat acidulous to the taste—thus the common names, sorrel-tree and sourwood.

The tree, as shown on the map (fig. 1), is distributed east of the Mississippi River from New Jersey to Illinois and southward to the Gulf of Mexico and northern Florida. The map is based on specimens examined cytologically (*diamonds*) and on those in certain herbaria (*circles*).[2]

TABLE 1

Stations at which O. arboreum was found to have 24 chromosomes at leaf metaphase

West Virginia	Georgia
Monongalia County: Morgantown	De Kalb County: Emory University
Nicholas County: Powell's Mountain	Rabun County: Pine Mountain
Raleigh County: Beckley	Alabama
Virginia	Bibb County: Pratt's Ferry Bridge
Bedford County: Coleman's Falls	Clarke County: Jackson
Buckingham County: Dillwyn	Cullman County: Cullman
Campbell County: Rustburg	Morgan County
Charlotte County: Keysville	Tuscaloosa County: Tuscaloosa
James City County: Williamsburg	Wilcox County: Pine Hill
Pulaski County: Dublin	Mississippi
Washington County: Abingdon	Jackson County: Ocean Springs
Wise County: Coeburn	Tennessee
North Carolina	Polk County: Ducktown
Cherokee County: Murphy	Putnam County: Baxter
Jackson County: Dillsboro	Sevier County: Elkmont
Madison County: Hot Springs	Kentucky
McDowell County: Lake Tahoma	Harlan County: Cumberland
South Carolina	McCreary County: Whitley City
Greenville County: Marietta	
Oconee County: Upper Whitewater Falls	

During the summers of 1940 and 1941, as part of a broad program for cytogeographic analysis of North American plants (see Baldwin 1942), the writer, in routine fashion, made chromosome counts for thirty-one collections

[1] Papers from the Department of Botany of the University of Michigan, No. 795. Supported by Faculty Research Fund, Projects No. 540 and 569.

[2] Gray Herbarium, United States National Herbarium, and herbaria of the Field Museum of Natural History, New York Botanical Garden, Missouri Botanical Garden, Cornell University, West Virginia University, Duke University, University of Michigan, University of Tennessee, and University of Georgia. The writer appreciated the privilege of access to these specimens.

of *O. arboreum* from representative stations in the specific range. He also examined cytologically trees growing at The Blandy Experimental Farm in Virginia. The tree, being quite distinctly different from other Ericaceae, is easily recognized from a rapidly moving automobile, so the finding of mate-

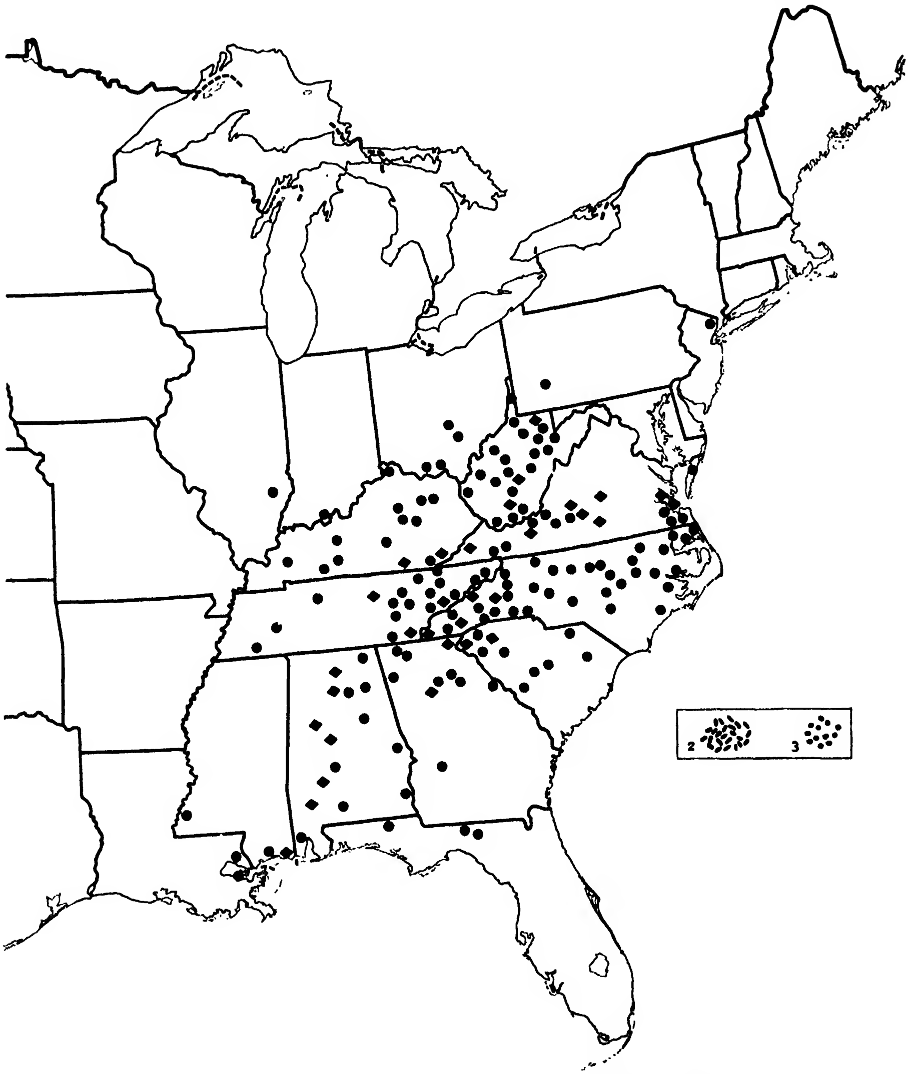

FIG. 1. Distribution of *O. arboreum* based upon herbarium specimens (circles) and upon specimens cytologically analyzed (diamonds). FIGS. 2–3. Mitotic metaphase in leaf cell: $2n = 24$. Meiotic metaphase in pollen mother cell: $n = 12$. *ca.* × 650.

rial is easy. Young leaves are produced from spring to fall, and from them leaf smears are readily made. Flowers in many developmental stages are present in a single inflorescence, and good aceto-carmine smears of anthers

are made without difficulty. Moreover, the writer had found intraspecific chromosome-number races in certain other species characteristic of the Appalachian system,—e.g., *Galax aphylla* L. and *Sedum ternatum* Michx.,—and considers that many cases of such polyploidy will be discovered by intensive cytogeographic surveys of species.

However, the chromosome number of *O. arboreum* was not observed to vary. At mitotic metaphase in leaves the $2n$-number is 24 (fig. 2); at meiotic metaphase in anthers the n-number is 12 (fig. 3). The chromosomes are uniformly small and of a similar morphology. Stations for wild plants investigated cytologically are listed in table 1 and, of course, designated on the map.

The conclusion to be drawn from this study is obvious: According to the data at hand *O. arboreum* has throughout its geographic range a $2n$-number of 24 chromosomes.

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THE GENUS DELPHINIUM IN NORTH AMERICA: SERIES ECHINATAE OF SUBSECTION SUBSCAPOSA, AND MISCELLANEOUS NOTEWORTHY SPECIES

JOSEPH EWAN

The present small series of larkspurs is marked phylogenetically as a series in probable advanced position among the North American species. It is characterized at once by its distinctive echinate seed coats and by its well-defined Californian distribution along the eastern rim of the Great Valley. The single species with its three component subspecies making up this series inhabits the warm interior foothills of the Sierra Nevada of California, a region otherwise noted as one of remarkable floristic content. *Delphinium hanseni* lives in the same region with such distinctive endemics as the genus *Carpenteria, Collomia rawsoniana, Arctostaphylos myrtifolia, Githopsis pulchella, Wyethia elata* and *W. reticulata,* and the remarkable and unique species *Delphinium purpusi.*

The origin of this well-marked series Echinatae of the subsection *Subscaposa* cannot be determined with any certainty at this time. Though the echinate seeds of *Delphinium hanseni* are unique among the Californian larkspurs, the other characters of flower, follicle, and habit are not at variance with the Californian members of this large subsection. *Delphinium hanseni* may have had its origin conceivably from *D. hesperium,* its closest ally among the related series. Furthermore, what I believe are natural hybrids are fairly frequent between these two species. Cytogenetic studies may yet demonstrate the basis for such suspected hybridism.

The author is again indebted to many curators for continued assistance in various ways. The material to be found in the herbaria enumerated in an earlier paper (1936) has been used as the basis for the present paper. In addition large series from the Academy of Natural Sciences, Philadelphia, the Rocky Mountain Herbarium, Laramie, and the growing collections being assembled by the author through field work and the generous courtesy of several correspondents, have been studied. In particular valuable material has been received from Mr. Milo Baker, Dr. Lincoln Constance, Dr. C. L. Hitchcock, Dr. R. F. Hoover, Mr. John Thomas Howell, and Mr. J. W. Thompson. A visit to the Herbarium Greeneanum at Notre Dame University during February, 1940, was particularly profitable. The *Delphinium* collection there has more critical sheets than any other North American herbarium examined, notwithstanding its relatively small size. Several species of great rarity in the largest collections are represented among E. L. Greene's collections. I am indebted to Dr. Theodor Just for his courtesies. The Parry Her-

barium at Iowa State College, Ames, under the curatorship of Dr. George Goodman, is rich in historic collections. The sheets must often be given individual study against Parry's scattered published annotations and floristic lists, however, for they commonly are very imperfectly labelled.

HISTORICAL NOTE

Though Thomas Bridges, William Lobb and J. M. Bigelow traversed the Sierra foothills inhabited by *Delphinium hanseni* during the meridian decades of the nineteenth century, I have not seen any specimens of this larkspur taken by them. Indeed this *Delphinium* seems not to have been taken until the final decades of the last century when Greene, Curran (Mrs. Mary Katherine Layne Curran Brandegee at her death), Congdon, Hansen, Jepson, and Davy penetrated these foothill valleys and Digger Pine (*Pinus sabiniana*) slopes. Greene first distinguished the plant as a variety of *Delphinium hesperium.* At this time (1891) Greene had not seen fruiting specimens, but he was quick to propose (1896) the variety for species status when he saw the "marked peculiarity of the seeds" demonstrated by fruiting specimens obtained by Alice Eastwood. At this time (1896) he described in a rather indefinite manner "the variety or subspecies" *arcuatum* without assigning a representative collection or so much as delimiting it with a blanket distributional phrase.

One of the most prophetic statements regarding *Delphinium hanseni* was that of Dr. Anstruther Davidson when he wagered that though Asa Gray in the Synoptical Flora (**1**: 49. 1895) had placed the hispid-hirsute-leaved larkspurs of central California under *D. hesperium* he felt all the hispid forms would prove to be *D. hanseni* when the seeds were examined and the species better known. In my present study this wager of Dr. Davidson's (1908) has been borne out and, apart from individuals of *D. variegatum* which occur infrequently in the range of *D. hanseni,* no other hispid-hirsute-leaved larkspur occupies the central Californian Sierra foothills. This is a fact of value in the identification of imperfect specimens from this region.

It is appropriate historically that a *Delphinium* so distinctive of lower-middle elevations of the Sierra foothills should bear the name of a collector, George Hansen (1863–1908) who so eagerly botanized over this region. Jepson has written of Hansen in the Dictionary of American Biography and also, with portrait, in Madroño (**1**: 183–185. 1928).

CONTINUED USE OF THE CATEGORY SUBSPECIES

Since formulating my views on the desirability of using the subspecies category (1936, p. 329) I have continued to scrutinize the practice. I remain confident of the enduring value of the use of the term subspecies to replace the more inexact and variously used term variety. It is unfortunate that

several authors see fit to recast the names given by workers using the subspecies category into new combinations under "variety." Nor do I see any substantial progress in the reciprocal practice of somewhat automatically changing varietal names to subspecies as new combinations. To obviate this persistent confusion, which cannot but reflect discredit upon systematic botany, it seems to me desirable to adopt the straight trinomial when referring to the rank below that of species, in the manner established many years ago by Coville, Piper and Abrams. Thus whether a given name was proposed as a subspecies or variety becomes inconsequential beside the direct practical manner of referring, for example, to *Delphinium hanseni arcuatum.*

Along with this usage may stand another device to remove nomenclatural difficulty today. Authors have treated the populations of the nomenclatorial type of a species as subspecies or variety *typicum.* Again some authors have used for the good reason of avoiding the concept of "biologic type" the terms *originarium, genuinum,* or *verum,* indicating that it is the subdivision which includes the type of the specific epithet. There has been some use of the specific epithet unaltered (e.g., *Stachys recta* subsp. *recta*) among European systematists but apparently no use of this clear and direct device has been made by taxonomists in this country.

Benson has pointed out to good effect (Bull. Torrey Club **68**: 160. 1941) that "while the validity of the philosophy behind the use of the formal name 'var. *typicum'* is admitted, it is [his opinion] that publication of formal trinomials for typical varieties is unnecessary." In the present proposal there need be no formal publication of typical varieties but the convenience of using the repetitious trinomial for precision of reference to the typical subspecies is accomplished. In this and future papers I shall follow this practice, found workable by the systematic zoologists, and embraced in Article 56, Recommendation XXXV, International Rules of Botanical Nomenclature (Jour. Bot. suppl. 1934).

SYSTEMATIC TREATMENT

Subsection *Subscaposa* (Huth) Ewan, Bull. Torrey Club **63**: 330. 1936.

Series **Echinatae** ser. nov. Semina obpyramidalis et acutis, perspicue integumentis echinatus cum imbricati-squamellatis.

Perennials of subcapose habit with seeds distinctly scaly-echinate and bur-like with loosely imbricated prominent thin scales or processes on the seed face. TYPE SPECIES of series Echinatae: *D. hanseni* (Greene) Greene.

KEY TO THE SUBSPECIES OF DELPHINIUM HANSENI

Stems short (30 cm. tall or less), stout; leaves shaggy-white pilose; raceme dense .... 1c. *D. hanseni kernense*

Stems taller (40–90 cm. tall), often strict but scarcely stout; leaves glabrate to hirsute or pubescent along veins but not pilose; raceme dense to loosely interrupted.

Flowers dark blue to purple, the racemes often dense or compact, frequently narrow or spiciform; lower leaves generally withering at flowering time .... 1a. *D. hanseni hanseni*
Flowers whitish or pink, seldom blue, the racemes commonly loose or interrupted, not at all spike-like; lower leaves often green and conspicuous at flowering time .... 1b. *D. hanseni arcuatum*

1a. D. HANSENI (Greene) Greene,[1] Pittonia **3**: 94. 1896, based on *D. hesperium* var. *Hanseni* Greene, Fl. Franciscana 304. 1892, in turn based on *Geo. Hansen* coll., 1891, from Amador Co., Calif., without definite locality. TYPE (Notre Dame Herb. without accession number) studied. Another sheet (N. D. Herb. 3413), Eldorado, Calaveras Co., VI 1889, *Greene*, is a good paratype; though not referred to by Greene, unmistakably involved in Greene's original description. Jepson records (Fl. Calif. 1: 523. 1915) a verbal statement made in 1896 by Prof. Greene to the effect that "the best type of it is Davy's 1326, Calaveras Co." Greene here likely had in mind the ideal type representative of the population in nature as he knew it.

Slender virgate perennial 50–90 cm. tall, arising from a rather short slender taproot, the stems greenish or fuscous above and darkening below, rather uniformly puberulent throughout with longitudinal glabrous lines, sometimes pilose at the base with white hairs; leaves not very conspicuous and withering at flowering time, mostly basal, dimorphic, the lower rhomboid with an ample little-divided blade, hirsute, 4–9 cm. wide, the upper divided to midrib into 3 or 5 narrow primary segments, the blade about ½ as broad as that of the lower leaves, much less pubescent or even glabrate, the petioles long, even the uppermost more than twice as long as the blade, all usually pilose or the upper merely puberulent; raceme rather compact, narrow-spiciform, elongating relatively little in fruit, the flowers numerous, commonly dark blue-purple or again (especially among colonies on the margins of its range) pinkish or even white and fading pink, short-pedicellate, the pedicels slender, ascending, shorter than the spur; sepals oblong, bluntish or rounded, commonly hoary with a median band of pubescence, 6–8 mm. long, spur curving, 7–10 mm. long, slender, acutish; follicles erect, nearly oblong, 10–14 mm. long, with slender moderately divergent filiform cusps, puberulent, a little venulose, borne on strictly ascending pedicels commonly appressed to rachis; seeds obpyramidal with a flat summit, silvery white, echinate, the faces with imbricate scale-like processes.

Often large colonies of 20–200 individuals in adobe or loamy soils of open hillsides, oak clearings or margins of cultivated fields in the Blue Oak-Digger Pine association, at 1500–3660 feet, but infrequent above 2500 feet. Sierra Nevada foothills from Butte Co. south to Madera Co., California, where plants somewhat intermediate between this and subspecies arcuatum occur. It spreads out upon the floor of the Great Valley on alkaline plains in Merced Co. April–May.

Representative material: CALIFORNIA—BUTTE: Bidwell Park, *Heller*

[1] It is the author's express intent to avoid *formal* establishment of any name for *typical* subspecies, i. e. "*D. hanseni hanseni* nomen nov.," but to indicate by such usage that the typical phase of the species is intended. Technically such trinomials should be credited to Greene; the trinomial is wholly unnecessary except when the component subspecies of the species is in question. The aim is to give a definite name of reference without involving nomenclature.

13625; Chico, *Ed. Palmer 2076;* Butte Co. foothills, 1897, *Bruce.* SUTTER: Marysville Buttes, *Heller 1914.* NEVADA: Limekiln Rch. near Wolf Creek, *Eastwood 3441.* PLACER: Auburn, VI 1886, *Shockley;* Roseville, *Congdon.* AMADOR: Ione to Clement, 2000 ft., *Belshaw 805;* Agricultural sta., 2000 ft., VI 1893, *Hansen 104;* Irishtown, 1500 ft., 18 VI 1896, *Hansen 104* (Pomona Coll.—note same coll. no.). CALAVERAS: Copperopolis, *Tracy 5576;* Table Hills near Sheep Rch., *Davy 1608;* Milton, *Davy 1321;* Wallace, 1914, *McMurphy;* Mokelumne Hill, *Blaisdell.* TUOLUMNE: 5 mi. e. LaGrange, *Hoover 2144* (fresh fls. white); Hetch-Hetchy, 3660 ft., *A. L. Grant 1267* (Jepson Herb.). STANISLAUS: Knights Ferry, *Hoover 1031;* LaGrange, *Hoover 959* (fls. light pink). MERCED: 5 mi. s. Merced, *Hoover 555* (fls. white or drying pale blue); 10 mi. s. Merced, *Hoover 556* (fls. dark purple, cf. his 555); 3 mi. ne. Merced Falls, *Belshaw 2027* (fls. pale blue, seeds lightly echinate). MADERA: Daulton Creek, n. Madera, *Hoover 904;* Madera, *Eastwood 12609.*

George Hansen wrote Prof. Greene that he had "never found [*D. Hanseni*] below 1500 feet [elevation] nor above 1800 feet" (mss. note in Greene Herb.). We now know its altitudinal limits to be from 1500 to 3660 feet, though it is most characteristic of the lower elevations. Helpful notes on Hansen's little-known collecting localities may be found in Jepson's biography in Madroño (**1**: 184). The range of *D. hanseni hanseni* and of *D. hanseni arcuatum* are mutually exclusive except for Madera County, the zone of overlap of these two subspecies. Here some individuals are difficult to refer to one subspecies or the other with certainty. A single collection, Lindsey, Tulare Co., 1925, *Harter* (Calif. Acad.), has been seen which falls geographically far beyond the general range of typical *D. hanseni.* This collection has the dark blue-purple flowers and is in every way typical of the more northern plants.

There is a small-flowered race (sepals 5–7 mm. long, paler blue, the spur straight) in Amador and Calaveras counties which deserves some study. In fact most of Hansen's collections represent this race. Along the margins of the Great Valley Dr. Hoover has detected some interesting individuals. One collection seems clearly to be a hybrid between *D. variegatum* and *D. hanseni,* the former species occurring in the same colony (*Hoover 956* and *956a,* LaGrange, Stanislaus Co., 13 IV 1936). On May 2nd, of the same year, some plants of the colony exhibited seeds like *D. hanseni* but the processes were more or less scattered and not closely studding the seed coat. Some plants otherwise identical had bullet-shaped seeds with a "loose cellular dirty-white coat without projections," suggestive of *D. variegatum.*

1b. D. HANSENI var. ARCUATUM Greene, Pittonia **3**: 94. 1896, based on undesignated type. LECTOTYPE: Yosemite Valley, VII 1896, ***Jepson sine numero*** in Jepson Herb. Paratype: Little Yosemite, VII 1875, ***McLean*** in Univ. Calif. Herb. No sheets in the Greene Herb. at Notre Dame Univ. offer a type basis.

Slender erect or straggling perennial 40–80 cm. tall of varying habit,

arising from a rather long, woody, sometimes stout, much-branched taproot, the stems greenish, bluish or violescent, not strictly virgate, sometimes laxly spreading, puberulent or glaucescent above, pubescent toward the base; leaves green and evident at flowering time, almost altogether basal, not distinctly dimorphic since the cauline commonly much reduced, inconspicuous or wanting, the lower irregularly pentagonal or rhomboidal, the blade ample, 4–6 cm. across, with 3 to 5 unequal broad primary divisions, the ultimate divisions few, variable, blunt to acute, glabrate except for the long spreading hairs along the veins and on the margins (or when the blade is only 2–3 cm. wide then hairy-pubescent on both surfaces and the segments involute), the petioles always white-pilose or ciliate; racemes loose, elongating in fruit, the flowers few, commonly greenish-white tinted with green or red umbo, often variable in the same colony, the pedicel about equaling the spur; sepals oval or nearly oblong, obtuse or barely acute, puberulent but without a median band of pubescence, often umbonate, (7 to) 9–10 mm. long, the spur often curving upwards, rather stout, 7–10 mm. long; immature follicles indicate the same characters as *D. hanseni hanseni,* the fruiting racemes 15–20 cm. long; ripe seeds unknown.

Small colonies in thin rocky or sandy soils among boulders and shrubs, commonly in the shade, sometimes about waterfalls and springy seeps at higher elevations, generally in Blue Oak-California Buckeye association, less often with *Libocedrus* along the streams, from 1500–4000 feet. May.

Representative material: CALIFORNIA—MARIPOSA: foot of Yosemite Falls, *Chandler 1144;* Mariposa, 1883, *Congdon.* MADERA: Raymond, *Eastwood 12528;* Fresno Creek, *Hall 10044.* FRESNO: Dunlap, *Jepson 2758;* 1 mi. nw. Squaw Valley, 1600 ft., *Constance 2226.* TULARE: Porterville, 1900, *Dudley;* Tule R., 1897, *Dudley;* Pine Flat near Calif. Hot Sprs., *Moxley 579;* Lemon Cove, *Bacigalupi 1189;* Springville, *Purpus 5049;* Kaweah R. Basin, *Hopping 22.* KERN: Erskine Creek, *Purpus 5016* (USNH); Kern R. Canyon, *Peirson 7316, Abrams 11987;* Caliente Creek Canyon, *Keck 2264.*

Delphinium hanseni arcuatum shows considerable variability in its characters, even within the same colony. It represents quite uniformly the pale-flowered phase of this species. One collection, *Bacigalupi 2344* (Ewan Herb.), from 5 mi. s. Fairview, Lower Kern R. Canyon, 3200 ft., Tulare Co., is remarkable for the bright blue flowers in an elongated sparsely flowered raceme. The leaves are typical of *D. hanseni arcuatum* but the fact that *D. purpusii* grows in the same region (*Bacigalupi 2342,* Lower Kern R. Canyon, 3520 ft.) suggests the possibility of hybridization with that species. The flower color of *Bacigalupi 2344* is unlike that seen among any of several score collections of *D. hanseni.*

Some collections are intermediate between the two subspecies *D. hanseni hanseni* and *D. hanseni arcuatum.* These plants which combine characters are numerically few among the total series studied and come from both extremities of the range of *arcuatum.* These intermediates may be illustrated by three combinations: *a.* dark blue and whitish flowers in same colony, as Mariposa, 1895, *Congdon* (Dudley Herb.) and Kings R., Fresno Co., 1923,

Duncan (Dudley Herb.); *b.* subsp. *Hanseni* as to flower color and inflorescence but subsp. *arcuatum* in leaf characters, as Greenhorn Range, Kern Co., *Hall & Babcock 5065* (Univ. Calif. Herb.). This collection was placed in *D. hanseni kernense* with some question by Davidson; *c.* variation in shape of follicle within same collection: Raymond, Madera Co., *Eastwood 12528* (Pomona Coll.).

1c. D. HANSENI var. KERNENSE Davidson, Muhl. **4**: 37. 1908, based on *Hasse & Davidson 1703* from "dry sunny slope," Mt. Cummings, Tehachapi Range, Kern Co., Calif. TYPE (Los Angeles Mus. Herb. 3976) studied.

Stout, strict, simple-stemmed perennial 20–35 cm. tall, arising from a cluster of deep, stout, woody roots, the stem cyaneous, puberulent above, pubescent toward the base with short curling hairs; leaves chiefly basal, suborbicular or broadly deltoid, the ultimate segments bluntish or shortly acute, mucronate, shaggy-white pilose, especially along the veins beneath, thinly pilose above, 3.0–3.5 cm. long, 4.5–5.5 cm. broad, the petioles noticeably long, 3–4 or even 10 cm. long, pilose; raceme rather dense, short, 5–8 cm. long, the flowers pale bluish white, sepals ovate, 7–10 mm. long, recurved at tips, uniformly puberulent externally, about equaling the lower petals, the blade of petals rounded, scarcely emarginate, the spur short, 6–9 mm. long; follicles unknown.

Small colonies on grassy slopes among scattered shrubs of *Ceanothus* or *Arctostaphylos*, just below the thin forest of Yellow Pine. Dr. Davidson reports that Mt. Cummings was reached from the east, camping near "Dr. Minne's Mine" about seven miles west of Tehachapi station (see Muhl. **4**: 65–68. 1908). Bauer does not record this *Delphinium* for the Tehachapi Range (Bull. S. Calif. Acad. Sci. **29**: 96–99. 1930).

Other collections examined: CALIFORNIA—KERN: Caliente Creek, *F. Grinnell 12* (USNH); Bisses sta., *Dudley 469* (USNH, Dudley Herb.); near Bena, Tehachapi, 1000 ft., IV 1928, *H. L. Bauer* (Univ. S. Calif.).

When characterizing *D. amabile pallidum* I then (1936, 337) placed *D. hanseni kernense* there as a doubtful synonym of that subspecies of the Mt. Pinos region to the southwest of the Tehachapi Range. At that time I had seen but one other collection aside from Davidson's type. Moreover, the type collection of *kernense* is singular in some respects; of course it is an early season collection. Though the seeds are still unknown to me I am restoring this subspecies to its original alliance with *D. hanseni*. All of the collections cited by me under *D. amabile pallidum* are properly that species. Though these local Kern County delphiniums agree in having a stout strict habit, nearly the same pallid flower color, and flower size, they may be distinguished as follows:

Raceme densely flowered; flowers bluish white; lower petals scarcely emarginate; leaves shaggy-white pilose .... *D. hanseni kernense*
Raceme rather open to densely flowered; flowers whitish; lower petals distinctly emarginate; leaves generally glabrous .... *D. amabile pallidum*

MISCELLANEA OF VARIOUS NOTEWORTHY DELPHINIUMS

For purposes of citation in current floristic surveys and manuals it is desirable to append here the following notes relative to species belonging to other series or subsections of the genus:

1. **D. bakeri** Ewan, sp. nov., based on *Milo S. Baker 9489* from Coleman Valley, Sonoma Co., Calif., V 1939. TYPE (Ewan Herb. at Univ. Colo.) and isotype in M. S. Baker Herbarium, Santa Rosa Junior College, Calif.

Slender comely perennial 50–65 cm. tall, arising from a cluster of thickened tuberiform fleshy roots, the stems erect, rather fistulous, dark reddish below, subglabrous with thinly scattered hairs, leafy throughout, the leaves decorative, not withering at flowering time, ample, rather thin, the blades of the principal leaves Ranunculus-like, 6.5–7.5 cm. wide, shallowly pentafid, the primary segments of broadly cuneate lobes, these again crenately toothed into short-apiculate or mucronulate teeth, the upper cauline leaves reduced and more deeply 5- or 3-divided, all glabrous or nearly so, a little paler beneath, the petioles slender, subamplexicaul by a flaring base, the base fuscous-hirsute with spreading golden (? glandular) hairs; racemes short-oblong, rather loosely few-flowered (5–15), the flowers on ascending or spreading pedicels 8–20 mm. long at anthesis, their sepals dark blue or purplish but bright, lance-ovate, acute, 11–13 mm. long, glabrous, a little shorter than the slender nearly straight spur, the upper petals, oblique, somewhat crisped, white, the lower petals erosulate or irregularly notched, the blade oblong, blue-purple, inconspicuously villous with a few whitish hairs; immature follicles slender, suberect, glabrous, venulose, with rather prominent cusps; seeds unknown.

Herba perennis caulibus erectis gracilibus, 50–65 cm. altis, e radice grumosa tuberiforma, simplicibus, interdum fistulosis, furvis, subglabris; foliis consimilis Ranunculo, exmarcidis, amplis, interdum tenuis, 6.5–7.5 cm. latis, pentapartitibus, segmentis lato-cuneatis divisis crenatis et breve apiculatis vel mucronulatis, caulibus superioribus reductis, profunde pentapartitibus vel tripartitibus, omnino glabris vel prope, pallidis subter, petiolis gracilibus subamplexicaulibus lucidis hirsutisque atque (?) glandulosis; racemis suboblongis, floribus 5–15, inferioribus remotis, pedicellis divaricatis vel ascendentibus 8–20 mm. longis, sepalis atrocaeruleis tamen lucidis lanceo-ovalibus acutis, 11–13 mm. longis, glabris, calcar subrectis gracilibusque longioris quam sepalis, petalis superioribus obliquis, crispulatis, candidis, inferioribus oblongis, erosulatis, atrocaeruleis, vix villosulis; folliculis immaturis suberectis et gracilibus glabrisque venulosis, cuspide prominentiore ornatis; seminibus ignotis.

Delphinium bakeri is a remarkable species. Mr. Milo S. Baker writes (3 IV 1940) that he has known of its existence for eight to ten years but has never seen it elsewhere. The species grows today along fence rows and in heavy low brush but was formerly much more abundant than now at Coleman Valley, growing where there are now grainfields. The ranch where it grows "has been used as a dairy ranch all these years. It is now nearly extinct on this ranch at least. I thought," says Mr. Baker, "it rather closer to *D. trollifolium* than to any other local species. However it does not seem to be poisonous to stock as is that species." Coleman Valley, west of Occidental, at an

elevation of from 400 to 500 feet, has a colony of *Ranunculus orthorhynchus,* which, according to Milo Baker, is otherwise not known from Sonoma County but does occur in Lake County at 2300 feet. Also growing with this larkspur is *Potentilla elata,* as Mr. Baker says, "where one would expect *P. californica.*" Again, *Potentilla elata* occurs near *Ranunculus orthorhynchus* in Lake County. Keck, under Horkelia, records (Lloydia **1**: 84. 1938) three collections of this *Potentilla* for Sonoma County. The distribution of these three species belonging to two plant families suggests that they are relicts in the present flora of California's North Coast Ranges. It will be particularly interesting to discover *Delphinium bakeri* in Lake County! Prints of the unique type collection will be distributed to several herbaria and to other interested persons in the hope that this fast disappearing larkspur may be saved from extinction.

2. **D. caprorum** Ewan, sp. nov., based on *J. W. Thompson 15206* from Goat Rocks, 7000 ft., Cascade Mts., Wash. TYPE (Ewan Herb. at Univ. Colo.), isotypes to be distributed.

Showy stout low subsucculent perennial 15–20 cm. tall, arising from a slender basal stem upon a cluster of cord-like deep-seated roots; stem simple, glabrous, cyaneous or pale; leaves predominantly basal, orbicular in outline, 2.5–5.5 cm. wide, tripartite, the lateral primary segments again ternate, the ultimate segments oblong, approximate or close-set or even oval and overlapping, all obtuse, mucronulate, the veins prominent, the lower leaves on spreading petioles 10–12 cm. long; racemes showy, oblong, rather loosely 7–15 flowered, tardily elongating below; flowers on ascending pedicels, lightly villous distally, large, 15–24 mm. long, their sepals oval to ovate, obtuse or barely acute, dark blue or purplish, rather dull externally, finely dark venose, 14–17 mm. long, 7–11 (or 13!) mm. wide, glabrous or with a light band of puberulence medianly, the spur about equaling sepals, proportionately slender, curving at the tip, acute, the upper petals clavate-oblong, white, darkly venulose, the lower petals erosulate and shallowly retuse, the blades suborbicular, dark blue-purple, with a central floc of white curling hairs; follicles oblong, nearly parallel, only a little spreading, 11–14 mm. long, hirsutulose dorsally, rather faintly venulose; seeds oblong or bullet-shaped 2 mm. long, jet-black with a narrow white wing on the weak angles.

Herba humilis speciosis perennis caulibus rigidis subsucculentis 15–20 cm. altis, e radice valido funiforma, simplicibus, caesiis glabrisque; foliis imprimis ad caulis basim confertis, orbicularis, 2.5–5.5 cm. latis, tripartitibus, segmentis lateralis contra ternatis, divisis oblongis vel approximatis etiam ovalis valvatisque, omnino obtusis, mucronulatis, venosis, foliis inferioribus petiolatis 10–12 cm. longis; racemis speciosis, oblongis, patentibus atque tarde elongatis; floribus 7–15, magnis, sepalis ovalis vel ovatis, obtusis, 14–17 mm. longis, 7–11 (vel. 13!) mm. latis, atro-caeruleis vel purpurascentibus, ab exteriore parte pallescentibus glabrisve, calcar subaequalibus, acutis, gracilis, petalis superioribus clavato-oblongis, albidis, inferioribus erosulatis atque retusis, suborbicularis, purpurascentibus, albo-villosulis; folliculis oblongis, subparallelibus vix divaricatisque, 11–14 mm. longis, ad

dorsum hirsutulosis, seminibus oblongis vel glandiformis 2 mm. longis, nigerrimis, impressis infirme angulatis similiter sordido-albidis alatis.

This species is probably highly localized. It grows on alpine rock slides or scree and is known only from Goat Rocks or Goat Mt., southeast of Mt. Rainier. It is unmistakably related closely to another little known endemic, *Delphinium glareosum* of the Olympics, from which it differs chiefly in characters of the inflorescence and flower size, as here tabulated:

Delphinium caprorum	*Delphinium glareosum*
Stems 15–20 cm. tall	*Stems* 22–38 cm. tall
Spur and sepals exteriorly lightly pubescent, not at all glandular	*Spur* and sepals exteriorly villose and more or less glandular
Pedicels a little villous just subtending the flower	*Pedicels* villous with conspicuous hairs, glandular for distal ½ of its length
Flowers 15–24 mm. long, very showy	*Flowers* 12–18 mm. long, moderately showy
Follicles hirsutulose dorsally	*Follicles* wholly glabrate
Based on type, *Thompson 15206*	*Based on* type, *Piper 2003* (Notre Dame Herb. 3334) and *H. E. & V. L. Bailey 293* (Ewan Herb.), ridge betw. Hayden Pass and Mt. Claywood, Mt. Olympus Nat. Mon.

This larkspur resembles *Delphinium glareosum* in its general habit, leaves, spreading pedicels and purplish-blue flowers. It is likewise a plant of scree slopes. *D. caprorum* is doubtless referred to by Piper (Fl. Wash. 280) when he places *Allen 146*, Goat Mt., under *D. bicolor*. That is not an alpine species, however, and differs in several particulars from either of these apparent relict species of Washington's older mountain summits.

3. D. NUDICAULE forma **elatium** (Thompson) Ewan, comb. nov., based on *D. nudicaule* var. *elatius* Thompson, Garden **19**: 234. 1881, in turn based on (?) garden plant, the "taller form with more leafy stems, the flowers rather longer with more slender spurs than in the typical state."

The English amateur gardener, William Thompson (1823–1903), drew the distinctions between typical *D. nudicaule* and forma *elatium* even before the date given above. In the periodical Garden, for June, 1873, vol. 3, p. 477, he itemized the observed distinctions as noted *in horto*. These should be given some field attention but at present there is no apparent phytogeographic significance in the distribution of forma *elatium*.

Typical *Delphinium nudicaule*	*D. nudicaule* forma *elatium*
Stems 3–6 dm. high, scarcely leafy	*Stems* 5–10 dm. high, leafy, giving a pyramidal aspect
Tubers rarely more than 5 cm. long	*Tubers* 12–20 cm. long, divergent
Flowers seldom more than 25 mm. long	*Flowers* 25–40 mm. long
Spur stout, short, curving	*Spur* slender, straighter
Petals more or less included	*Petals* rather distinctly exserted
Seeds numerous	*Seeds* few, "more than twice as large"

Representative collections: CALIFORNIA—MENDOCINO: Longvale, *S. W. Hutchinson 5587*. NAPA: Sarco Creek, 5 mi. above Napa, *Ewan 8662*. Forma *elatium* is apparently an established ecad, favoring brushy slopes associated

with *Ceanothus, Arctostaphylos, Symphoricarpos, Quercus garryana* and *Acer macrophyllum.*

4. D. PATENS subsp. **greenei** (Eastw.) Ewan, comb. nov., based on *D. greenei* Eastw., Bull. Torrey Club **28**: 674. 1901, in turn based on T. S. Brandegee coll., 29 V 1891, from Coburn Mills, s. Sierra Nevada, Fresno Co., Calif. TYPE (Calif. Acad. Sci. Herb. 232) studied.

5. D. PATENS subsp. **montanum** (Munz) Ewan, comb. nov., based on *D. parryi* var. *montanum* Munz, Bull. S. Calif. Acad. Sci. **31**: 61. 1932, in turn based on *Munz 6846,* Vincent Gulch, San Gabriel Mts., Los Angeles Co., Calif. TYPE (Pomona Coll. Herb. 18068) studied and type locality visited.

6. D. SCOPULORUM Gray, Fl. Wrightianae **2**: 9. 1853, based on *Charles Wright 842* from "mountain ravines near the Mimbres, New Mexico." Isotype (Phila. Acad. Nat. Sci.) studied and reproduced here as figure 1. This sheet is even a somewhat better representation than the TYPE (Gray Herb.).

More than thirty years ago E. O. Wooton expressed the view (Bull. Torrey Club **37**: 36. 1910) that "most of the material which has been passing as *D. scopulorum* is not very closely related to that species." The present author reiterated this view recently (Jour. Wash. Acad. Sci. **29**: 477. 1939). It is this prevalent misinterpretation that leads me to publish a photo of this apparently highly localized and distinctive *Delphinium.* There is at present no sound explanation to account for its erratic distribution. The only Colorado collection so far seen is *Parry 85* from "gravelly banks, dry ravines," July, 1862, with the generalization "Rocky Mts." From our knowledge of Parry's botanical activities in Colorado it can be placed more precisely as the Clear Creek watershed of central Colorado (cf. Ewan in April, 1941, *Trail and Timberline,* Colo. Mt. Club monthly). Identical in every respect is *Hall & Harbour 26* from the Rocky Mountains of Colorado, 1862. From the fact that these two collectors, Elihu Hall and J. P. Harbour, accompanied Parry during the season of 1862 is deduced this identity of specimens.

The relationships of *D. scopulorum* are with the species of the Mexican Sierra Madre Occidental, belonging to a wholly unrelated series from the more familiar and northern *D. occidentale* and *D. glaucum.* These northern *Delphiniums* are related to the Eurasian members of the Tribus *Elatopsis* of Huth. This phylogeny will be treated elsewhere. Meanwhile collectors are directed to true *D. scopulorum* in the hope that fruiting specimens will be taken from several stations in New Mexico and Colorado.

7. D. BURKEI Greene, Erythea **2**: 183. 1894, based on Burke coll., 1844–46, *sine numero,* from "Snake Country," that is, the Snake R. country of Idaho. TYPE at Kew; isotype should be looked for at Brit. Mus. Nat. Hist. *D. simplex* Dougl. ex Hook., Fl. Bor. Am. **1**: 25. 1829, based on Douglas coll. from "subalpine range, west of Rocky Mts., near the Columbia [R.]"; not *D. simplex* Salisb., 1796. Type (Kew Herb.) is pale-flowered form and was compared by

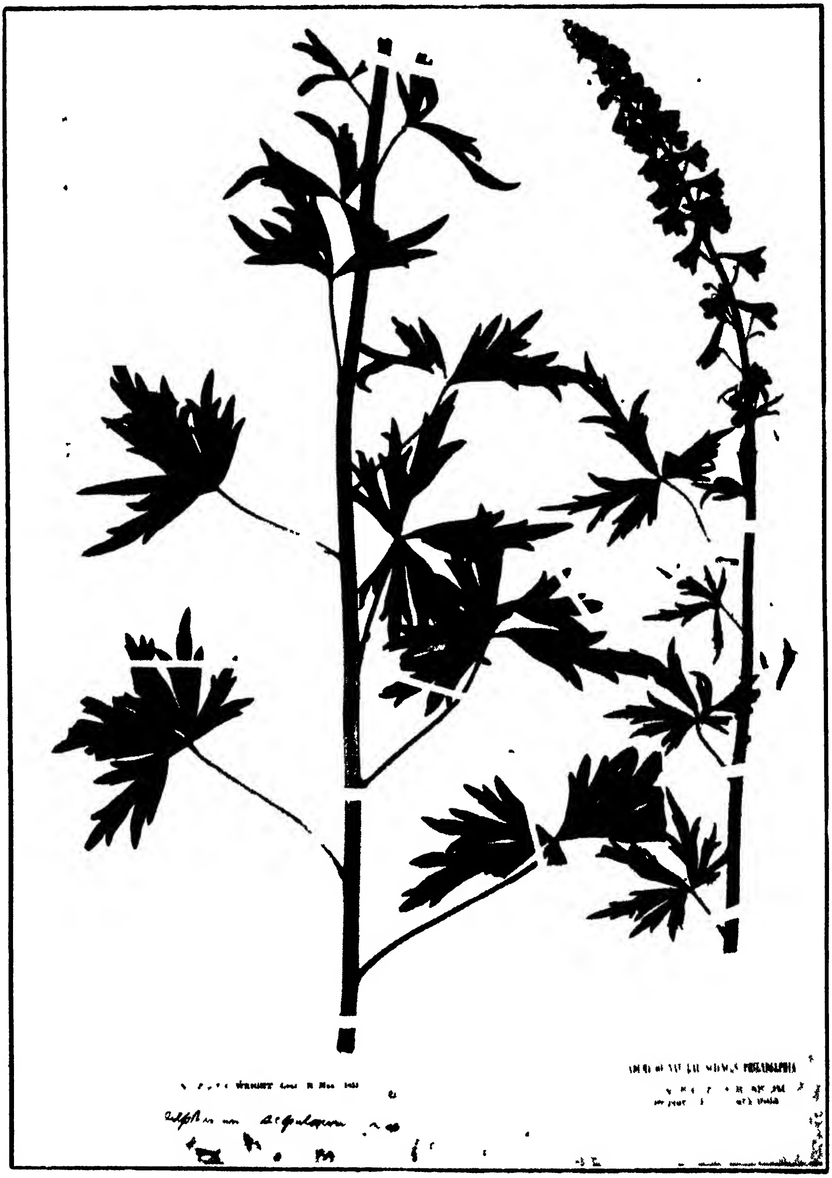

FIG. 1. Isotype of *Delphinium scopulorum* Gray.

Carl Epling with *Epling & Houck 9200,* Thatuna Hills, Ida., and annotated.

Delphinium burkei represents the larkspur still known by the preoccupied name *D. simplex* Dougl. Greene's specific name commemorates the British collector, Joseph Burke, who flourished between 1839 and 1846, botanizing in South Africa 1839–42, with Zeyher during 1840–41, and in North America 1844–46. Very little exact information exists regarding his

North American routes and movements (cf. Britten and Boulger, Biog. Index Brit. and Irish Bot. ed. 2. 1931). C. V. Piper, who in his Flora of Washington is usually informative on biographical items, does not mention Joseph Burke.

8. D. BURKEI subsp. **distichiflorum** (Hook.) Ewan, comb. nov., based on *D. simplex* var. *distichiflorum* Hook., London Jour. Bot. **6**: 67. 1847, in turn based on *Geyer 420* from high plains of Spokane and Nez Perces. Type in Herb. Hooker, Kew.

Those plants of *Delphinium burkei* with puberulent to wholly glabrous lower stems and leaves, the raceme densely compact, 30–45-flowered, is subsp. *distichiflorum*. It is apparently infrequent over the range of the species.

9. **D. umatillense** Ewan, sp. nov., based on *F. A. Warren 2089* from s. slope Madison Butte, Morrow Co., Oregon, 10 VIII 1937 and specimen in follicle, garden grown at Port Orchard, Wash., coll. 30 VI 1938. Type in Ewan Herb. at Univ. Colo.

Tall leafy perennial, 0.5–1.0 m. high, simple or branching from the base, arising from a woody fibrous clustered root, the stems green, slender, solid, puberulent and finely red flecked, rather evenly clothed with leaves, the leaves persistent at flowering time, ample, thin-textured, light green, the blades pentagonal, Acer-like flabelliform, 7–10 cm. wide, puberulent to glabrate, the primary segments cuneate, these again divided into lance-acuminate lobes, the ultimate segments acute; racemes oblong, rather open, 15–20-flowered, flowers 36–30 mm. long, on slender spreading pedicels, the sepals lanceolate, acuminate, puckered or crisped at tips, 11–13 mm. long, bright lively blue, yellowish below, the upper petals oblique rhomboid, entire and truncate, white on side, the lower petals uniformly light blue, the blades suborbicular, villous with curling white hairs; follicles nearly straight, erect or subparallel, venose, 14–16 mm. long, finely villous-pubescent, the cusps slender, erect; seeds dark, narrowly wing-margined, 2.0–2.5 mm. long, subtriquetrous.

Herba perennis caulibus erectis gracilibus, 0.5–1.0 m. altis, e radice tenui lignosa, simplicibus vel ramosis ad caulis basim, glabris vel puberulentis; foliis prorsus ad caulis distributis, pentapartitibus, segmentis cuneatis divisis lanceo-acuminatis, non filiformis ac brevioribus linearis, non mucronulatis; foliis caulibus aliquantulum reductis sed conspicuis, omnino puberulentis vel glabratis; racemis elongatis atque subpatentibus; floribus 26–30 mm. longis, pedicellis divaricatis, elatis, sepalis lanceolatis acuminatisve lucidis caeruleis et crispulis, 11–13 mm. longis, petalis superioribus rhomboidalis obliquisque integerrimis truncatisque, caeruleis in partim, albidis in partim, inferioribus suborbicularis omnino caeruleis et albo-villosis; folliculis suboblongibus, venosis, 14–16 mm. longis, villosulis, cuspide gracilibus, erectis, seminibus subtriquetris, 2.0–2.5 mm. longis, atrofuliginis impressis angulatis angustior similiter alatis.

Rocky mountain slopes and ridges, associated with *Juniperus occidentalis*, *Wyethia amplexicaulis*, *Balsamorrhiza sagittata* and species of *Artemisia*, of the arid Transition Zone of eastern Oregon. The type was taken in Umatilla National Forest on the upper slopes of the John Day River catchment basin, lying to the west of the Blue Mts.

The systematic position of this species can be best shown by a table contrasting *Delphinium umatillense* with *D. megacarpum,* known from this region, and *D. geyeri* of the plains and basins of more interior Idaho and eastward. Aside from their close morphology, then, these three related *Delphiniums* are likewise close geographic allies. (See table 1).

TABLE 1

Comparative morphology of three species of Delphinium

Characters	*D. megacarpum*	*D. umatillense*	*D. geyeri*
Stems	few-leaved, green cinereous-puberulent below	leafy, green, glabrous or nearly so	leafy, ashy pruinose-puberulent below
Basal leaves	suborbicular	Acer-like, flabelliform, pentagonal	Acer-like flabelliform, pentagonal
Base of primary segments of lvs.	cuneate	cuneate	subfiliform-linear
Base of petioles	conspicuously hirsute-ciliate	puberulent, not at all hairy	puberulent, not at all hairy
Flowers	dark-blue, rather densely racemose	bright lively blue, loosely racemose	bright lively blue, loosely to densely racemose
Sepals	"softly hirsute"	finely villous-puberulent, all lanceolate and acuminate	villose-puberulent, esp. medianly, the lateral sepals ovate, obtuse or apiculate
Spur	1⅓ as long as sepals, nearly straight	1½–2 times as long as sepals, curving and deflexed at tip (straight in bud)	1⅓–1½ times as long as sepals, slightly deflexed at tip or nearly straight (upcurving in bud)
Follicles	venose, puberulent, 18–25 mm. long, closely erect and parallel	venose, finely villose, 14–16 mm. long, erect	venose, finely hirsutulose, 13–15 mm. long, erect or a little divergent
Seeds	dark, narrowly wing-margined	dark, narrowly wing-margined	columnar-pyramidal, strongly margined
Data based on	Type, *Nelson & Macbride 1779* (Univ. Wyo. Herb.)	Type, *F. A. Warren 2089* (Ewan Herb. at Univ. Colo.)	*Nelson 7340, Ewan 11262, Ramaley 16372,* and *Cockerell s. n.* (all Herb. Univ. Colo.)

It may be suggested that *Delphinium umatillense* is of hybrid origin following the establishment of *D. megacarpum* in its present range in eastern Oregon and at a time when *D. geyeri* ranged farther to the west than at present. By reference to table 1 it will be seen that *Delphinium umatillense* is morphologically more or less intermediate between the two described species, combining leaf characters of *D. megacarpum* with the flower color of *D. geyeri.*

UNIVERSITY OF COLORADO
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NEW NORTH AMERICAN UMBELLIFERAE

MILDRED E. MATHIAS AND LINCOLN CONSTANCE

Hydrocotyle bowlesioides Mathias & Constance, sp. nov. Caules graciles procumbentes hirsuti; folia non peltata, subrotundo-reniformia, ad basim sinu praedita, petiolis exclusis 1–2 cm. longa, 1.5–3 cm. lata, marginibus breviter 5-lobatis, lobis obtuse triangularibus crenatis subaequalibus, utrinque hirsutis; petiolus gracilis, 1–12 cm. longus, praecipue ad apicem versus reflexo-hirsutus; pedunculi 0.2–1.2 cm. longi, quam folia multo breviores, axillares graciles sparse hirsuti; umbellae non proliferae, globosae, 2–10-florae; styli persistentes; stylopodium depressum; fructus sessilis, in ambitu ellipticus, circa 1 mm. longus, 1.5 mm. latus, in intervallis minute hispidulus, costis manifestis, acutis subaequalibus.

Stems slender, creeping, hirsute; leaves not peltate, roundish-reniform with a sinus at the base, excluding the petiole 1–2 cm. long, 1.5–3 cm. broad, the margins shallowly 5-lobed, the lobes obtusely triangular, crenate, subequal, hirsute on both surfaces; petiole slender, 1–12 cm. long, reflexed-hirsute especially above; peduncles much shorter than the leaves, axillary, slender, 0.2–1.2 cm. long, sparsely hirsute; umbels not proliferous, 2–10-flowered, globose; the styles persistent; the stylopodium depressed; fruit sessile, elliptical in general outline, about 1 mm. long, 1.5 mm. broad, finely hispidulous in the intervals, the ribs evident, acute, subequal.

TYPE: *A. F. Skutch 3573*, wet pasture, Vara Blanca de Sarapiqui, north slope of Central Cordillera, between Poas and Barba volcanoes, Costa Rica, alt. 1740 m., February, 1938 (United States National Herbarium, No. 1,643,724).

This species is most closely allied to *H. Torresiana* Rose & Standl. by virtue of its shallowly lobed leaves, short peduncles and sessile fruit, but is clearly separable by its fewer leaf lobes and hispidulous fruit. Both species are endemic to the Central Cordillera of Costa Rica, *H. Torresiana* occurring to the south of *H. bowlesioides,* on Volcan de Turrialba. The specific name of the new entity indicates its superficial vegetative similarity to certain members of the genus *Bowlesia* Ruiz & Pavon.

Arracacia Schneideri Mathias & Constance, sp. nov. Herba crassa caulescens, ramosa, e radice magna carnosa, circa 8–10 dm. alta, foliis inflorescentibusque puberulis; folia in ambitu ovata, petiolis exclusis 1–1.5 dm. longa, circa 1 dm. lata, ternata deinde 1–2-pinnati, foliolis ex lanceolatis ad ovatos, ad apicem acutis, ad basim cuneatis, ex distinctis ad confluentes, plerumque sessilibus, 1–4 cm. longis, 0.1–1.5 cm. latis, acute serratis, rhachibus foliisque puberulis; petioli crassi, 7–10 cm. longi, ad basim vaginis latis; folia caulina basiliaribus similia, vaginis late dilatatis; inflorescentia pauciramosa, pedunculis non-nihil crassis, 8–9 cm. longis, dense puberulis; involucrum bractea unica foliacea, obsoletum; involucella bracteolae paucae inconspicuae ex lanceolatis ad filiformes integrae, 3–10 mm. longae, quam flores et fructus longiora vel breviora; radii fertiles crassi, 12–20, patenti-adscendentes subaequales, 2–3 cm. longi, dense puberuli; flores eburnei, petalis obovatis; stylopodium indistinctum depressum, stylis tenuis patentibus, ovario glabro; car-

pophorum bifidum; fructus ovato-oblongus, 7–8 mm. longus, 3–4 mm. latus, glaber ad apicem attenuatus, costis prominentissimis acutis; vittae magnae solitariae in valleculis, 2 in commissura; semina sub vittis canaliculata facie sulcata.

Stout, caulescent, branching, from a large fleshy root, about 8–10 dm. high, the foliage and inflorescence puberulent; leaves ovate in general outline, excluding the petioles 1–1.5 dm. long, about 1 dm. broad, ternate, then 1–2-pinnate, the leaflets lanceolate to ovate, acute at apex, cuneate at base, distinct to confluent and usually sessile, 1–4 cm. long, 0.5–1.5 cm. broad, sharply serrate, puberulent on the rachis and leaflets; petioles stout, 7–10 cm. long, broadly sheathing at base; cauline leaves like the basal, with broadly dilated sheaths; inflorescence sparingly branched, the peduncles rather stout, 8–9 cm. long, densely puberulent; involucre wanting, or of a foliaceous bract; involucel of several inconspicuous, lanceolate to filiform, entire bractlets, 3–10 mm. long, longer or shorter than the flowers and fruit; fertile rays stout, 12–20, spreading-ascending, subequal, 2–3 cm. long, densely puberulent; pedicels spreading-ascending, 4–6 mm. long, puberulent; flowers creamy-white, the petals obovate; stylopodium indistinct and depressed, the styles slender, spreading, the ovary glabrous; carpophore 2-cleft to the base; fruit ovate-oblong, 7–8 mm. long, 3–4 mm. broad, glabrous, tapering at apex, the ribs very prominent, acute; oil tubes large, solitary in the intervals, 2 on the commissure; seed deeply channeled under the tubes, the face sulcate.

TYPE: *R. A. Schneider 1051*, rocky slope at timberline, Cerro Potosi, Municipo de Galeana, Nuevo Leon, alt. 12,100 feet, August 4, 1938 (Herbarium of the Field Museum of Natural History, No. 958,958).

This species is a member of an anomalous group in the genus, characterized by the possession of a depressed and inconspicuous stylopodium, instead of the usual conical one. *Arracacia Schneideri* appears to be most closely related to *A. vaginata* Coult. & Rose, of southern Mexico, from which it may readily be distinguished by its more compound leaves, with acute leaflets, its conspicuously inflated cauline leaf sheaths, and its much more numerous fertile rays.

Oxypolis Greenmanii Mathias & Constance, sp. nov. Plantae 18–24 dm. altae; folia ad phyllodia septata fistulosa reducta, 2.5–4.5 dm. longa, vaginis 8–12 cm. longis; pedunculi 5–15 cm. longi; involucra bracteae plures lanceolato-acuminatae, 10–20 mm. longae; involucella bracteolae subulatae, 2–8 mm. longae; radii 16–19, patentes, paullo inaequales, 2.5–5 cm. longi (per anthesin); pedicelli patentes, 3–15 mm. longi; flores atropurpurei; calycis dentes conspicui; fructus non visus.

Plants 18–24 dm. high; leaves reduced to hollow, septate phyllodes, 2.5–4.5 dm. long, the sheaths 8–12 cm. long; peduncles 5–15 cm. long; involucre of several lanceolate-acuminate bracts 10–20 mm. long; involucel of subulate bractlets 2–8 mm. long; rays 16–19, spreading, slightly unequal, 2.5–5 cm. long (in flower); pedicels spreading, 3–15 mm. long; flowers dark purple; calyx teeth conspicuous; fruit not seen.

TYPE: *A. W. Chapman*, Wewahitchka, Gulf County, western Florida, August, 1896 (Herbarium of the Missouri Botanical Garden, No. 787,696).

Oxypolis filiformis (Walt.) Britt. is apparently the nearest relative of this species. In general, *O. Greenmanii* may be distinguished from *O. filiformis* by its greater stature, much larger phyllodes, more numerous rays

and deep purple flowers. Fruiting material is needed before the relationship between the two species can be properly judged. The species is named for Dr. J. M. Greenman, who first recognized its distinctness.

Lomatium Hamblenae Mathias & Constance, sp. nov. Plantae acaulescentes vel brevi-caulescentes, 1–3.5 dm. altae ex tubere globoso, circa 1.5 cm. diametro, glabrae; folia in ambitu obovata, petiolis exclusis 4–12 cm. longa, univel biternata, deinde pinnata vel pinnato-lobata, divisionibus ultimis remotis linearibus 5–23 mm. longis, 1–3 mm. latis acutis apiculatis; petiolus 2–5 cm. longus, scariosus vel late scarioso-marginatus ad basim vaginatus; pedunculi folia excedentes; involucella bracteolae paucae, inconspicuae lineares lanceolataeve, distinctae connataeve quam pedicelli plurimum breviores; radii 2–8, adscendentes, 4–8 cm. longi inaequales; pedicelli 15–25 cm. longi, umbellulis 10–15-floris; flores claro-lutei; fructus lineari-oblongus, 4–7 mm. longus, 2–3 mm. latus, glaber, alis quam corpore multo angustioribus.

Plants acaulescent or short-caulescent, 1–3.5 dm. high, from a globose tuber about 1.5 cm. in diameter, glabrous; leaves obovate in general outline, excluding the petiole 4–12 cm. long, once or twice ternate, then pinnate or pinnately lobed, the ultimate divisions remote, linear, 5–23 mm. long, 1–3 mm. broad, acute, apiculate; petioles 2–5 cm. long, scarious or broadly scarious-margined, sheathing at base; peduncles exceeding the leaves, involucel of a few, inconspicuous, linear or lanceolate bractlets, distinct or connate, very much shorter than the pedicels; rays 2–8, ascending, 4–8 cm. long, unequal; pedicels 15–25 mm. long, the umbellets 10–15-flowered; flowers bright yellow; fruit linear-oblong, 4–7 mm. long, 2–3 mm. broad, glabrous, the wings much narrower than the body.

TYPE: *Frances G. Hamblen (Mrs. L. R.)*, on level scab-rock bench at Dry Falls, Grand Coulee, Washington, 1941 (Herbarium of the University of California).

The other bulbous yellow-flowered species do not seem to be closely related to the present species. The foliage, slender habit, nearly filiform, elongated pedicels and slender fruit point to an affinity with certain of the white-flowered species, notably *L. farinosum* (Geyer) Coult. & Rose.

LOMATIUM CILIOLATUM Jepson var. **Hooveri** Mathias & Constance, var. nov. Varietas speciei similis, multo gracilior, 1.5–3 dm. alta, dense scaberula; folia 6–13 cm. longa, divisionibus (lobis) ultimis linearibus, 1–10 mm. longis, non plus quam 1 mm. latis; petioli 3–7 cm. longi; pedunculi 8–25 cm. longi; radii 3–14, 3–10 cm. longi; pedicelli 3–8 mm. longi; fructus alae tenues.

Like the species, but much more slender throughout, 1.5–3 dm. high, densely scaberulous; leaves 6–13 cm. long, the ultimate divisions or lobes linear, 1–10 mm. long, 1 mm. or less broad; petioles 3–7 cm. long; peduncles 8–25 cm. long; rays 3–14, 3–10 cm. long; pedicels 3–8 mm. long; fruit wings thin.

TYPE: *Mathias 1298*, rocky hillside, 1.9 miles southeast of Napa-Lake County line, on road to Knoxville, Napa County, California, May 23, 1937 (Herbarium of the University of California).

Specimens examined: CALIFORNIA: just east of Lake County line on Williams–Clear Lake highway, Colusa County, May 2, 1941, *R. F. Hoover 4987*.

The pubescence, conspicuous bractlets and purple flowers relate this

variety closely to the species, of which it is apparently a more slender phase of lower altitudes. The foliage is very similar to that of the following species.

Lomatium Tracyi Mathias & Constance, sp. nov. Plantae acaulescentes, 1–3.5 dm. altae, ex glabris ad sparse scaberulo-puberulas, e radice primaria longa tenui; folia in ambitu ex oblongis ad ovata, petiolis exclusis 4–10 cm. longa, ternata deinde 1–2-pinnata, divisionibus ultimis ex linearibus ad oblongos acutis obtusisve, apiculatis, 1–7 mm. longis, 0.4–2 mm. latis; petiolus 2–5 cm. longus, scariosus ex toto vaginatus; pedunculi folia excedentes; involucella bracteolae ex oblonceolatis ad lineares acuminatae saepe petiolulatae, scarioso-marginatae, flores subaequantia; radii 4–9 (fertiles 1–6) inaequales, stricte adscendentes glabri vel scaberuli, 0.5–8 cm. longi; pedicelli 1–5 mm. longi, eorum pauci fertiles; umbellulae 10–15-florae; flores lutei; fructus ex oblongo-ovato ad ovalem, 6–10 mm. longus, 3–5 mm. latus, ad basim et ad apicem plerumque acutus, glaber, alis tenuibus quam corpore multo angustioribus; vittae obscurae.

Plants acaulescent, 1–3.5 dm. high, from a long, slender taproot, glabrous to sparsely scaberulous-puberulent; leaves oblong to ovate in general outline, excluding the petiole 4–10 cm. long, ternate, then 1–2-pinnate, the ultimate divisions linear to oblong, acute or obtuse, apiculate, 1–7 mm. long, 0.4–2 mm. broad; petiole 2–5 cm. long, scarious, wholly sheathing; peduncles exceeding the leaves; involucel of oblanceolate to linear, acuminate, often petiolulate, scarious-margined bractlets, about equalling the flowers; rays 4–9 (the fertile 1–6), strictly ascending, 0.5–8 cm. long, very unequal, glabrous or scaberulous; pedicels 1–5 mm. long, few fertile, the umbellets 10–15-flowered; flowers yellow; fruit oblong-ovate to oval, 6–10 mm. long, 3–5 mm. broad, usually acute at the base and the apex, glabrous, the wings thin, much narrower than the body; oil tubes obscure.

TYPE: *J. P. Tracy 12,895* serpentine gravel flats, especially under yellow pines, Grouse Mountain, Humboldt County, California, alt. 5000 feet, July 25, 1933 (Herbarium of the University of California).

Specimens examined: CALIFORNIA–HUMBOLDT: in open forest of Jeffrey pine, on red soil in serpentine belt, Horse Mountain, alt. about 5000 feet, June 20, 1936, *Tracy 7610;* under yellow pines, serpentine soil, Brannan Mountain, alt. 4000 feet, July 10, 1930, *Tracy 8869;* in gravelly soil, open situations, Mary Blaine Mountain, alt. 6400 feet, August 3, 1935, *Tracy 14,462;* red serpentine soil, French Camp Ridge, alt. 3500 feet, June 23, 1935, *Tracy 13,971.* TRINITY: near summit on Weaverville road, yellow pine forest, Hay Fork Mountain, alt. 3600 ft., June 30, 1923, *Tracy 6457;* openings among yellow pines, Burnt Ranch, alt. 1500 feet, June 28, 1923, *Tracy 6393.*

The group of species which comprises *L. caruifolium* (H. & A.) Coult. & Rose—with which the present species has been confused, *L. marginatum* (Benth.) Coult. & Rose and *L. ciliolatum* Jepson, has given a great deal of trouble to everyone attempting to construct a treatment of the genus. The separation of *L. Tracyi* will, we believe, make possible a more precise delimitation of *L. caruifolium,* and hence facilitate the erection of clearer specific boundaries within this group. From *L. caruifolium, L. Tracyi* differs in its lower stature, more slender habit, fewer rays, reduced involucel and sparser and usually narrower fruit, as well as in its higher altitudinal range and more northerly distribution.

Lomatium Peckianum Mathias & Constance, sp. nov. Plantae acaulescentes, 1–1.5 dm. altae, e scaberulis ad glabras, e radice primaria longa tenuissima; folia in ambitu ovato-oblonga, petiolis exclusis 2.5–5.5 cm. longa, ternata deinde 1–2-ternata, divisionibus ultimis e remotis ad confluentes, ex oblongis ad lineares, apiculatis, 1–18 mm. longis, 0.5–1.5 mm. latis; petiolus 2–4 cm. longus, laminae subaequalis, scarioso-marginatus ex toto vaginatus; pedunculi ex vaginis foliorum procedentes, folia excedentes, 6–10 cm. longi; involucella bracteolae obsoletae vel paucae, inconspicuae distinctae lineares subacuminatae subscariosae, pedicellis breviora; radii obsoleti vel 1–3, inaequales adscendentes, ad 3 cm. longi; pedicelli 2–7 mm. longi; plerumque una umbellula sessilis sterilis; umbellularum flores fertiles 1–7, steriles pauci; flores non visi; ovaria granulato-aspera; fructus oblongo-ovalis 2–13 mm. longus, 4–5.5 mm. latus, ex granulato-aspero ad glabratum, ad basim et ad apicem versus attenuatus, alis quam corpore minoribus dimido latitudine; vittae obscurae in commissura 6–8, in valliculis paucae.

Plants acaulescent, 1–1.5 dm. high, from a long, very slender taproot, scaberulous to glabrous; leaves ovate-oblong in general outline, excluding the petiole 2.5–5.5 cm. long, ternate, then 1–2-ternate, the ultimate divisions remote to confluent, oblong to linear, 1–18 mm. long, 0.5–1.5 mm. broad, apiculate; petiole 2–4 cm. long, about equalling the blade, scarious-margined, wholly sheathing; peduncles arising from a cluster of leaf sheaths, exceeding the leaves, 6–10 cm. long; involucel wanting, or of a few, inconspicuous, distinct, linear, subacuminate, subscarious bractlets, shorter than the pedicels; rays 1–3, ascending, obsolete to 3 cm. long, very unequal; pedicels 2–7 mm. long, the umbellets with 1–7 fertile flowers and a few sterile flowers, one umbellet usually sessile, sterile; flowers not seen, the ovaries granulate-roughened; fruit oblong-oval, 2–13 mm. long, 4–5.5 mm. broad, granulate-roughened to glabrate, narrower toward the base and the apex, the wings less than one-half the width of the body; oil tubes obscure, several in the intervals, about 6–8 on the commissure.

TYPE: *M. E. Peck 15,213*, dry sterile hillside three miles east of Blye, Klamath County, Oregon, June 25, 1927 (Herbarium of Willamette University).

The granulate-roughened young fruit would seem to relate this species to *L. Sandbergii* Coult. & Rose, which it does not otherwise closely resemble. It is perhaps more nearly allied to *L. nevadense* (Wats.) Coult. & Rose than to any other species, but is adequately distinct because of its ternately compound leaves, wholly sheathing petioles, inconspicuous involucel, few rays and more slender fruit.

We wish to express our gratitude to Miss Ethel K. Crum, Assistant Curator of the Herbarium of the University of California, for preparing the Latin diagnoses for this and other papers. We are also grateful to the curators of the herbaria mentioned for the opportunity to see the material cited. All collections listed, with the exception of the type specimens, are in the Herbarium of the University of California.

DEPARTMENT OF BOTANY, UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA

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SUPPLEMENTARY NOTES ON AMERICAN MENISPERMACEAE—II

B. A. KRUKOFF AND H. N. MOLDENKE

Through the courtesy of the curators of the two Brazilian botanical institutions mentioned below we were privileged recently to study their collections of the Menispermaceae. Most of these collections are not represented by duplicates outside of Brazil. They were found to be of considerable interest and are discussed in the present paper. The collections examined extend our knowledge of certain species previously known from incomplete material, extensions of ranges are noted for a number of species, and one species is described as new. No changes in the nomenclature are necessitated.

The species are arranged in the same order and the place of deposit of specimens is shown by the same abbreviations as in our previous papers (1, 2, 3). The following new abbreviations are used:

PG: Museo Goeldi de Historia Natural e Ethnographia, Pará, Brazil.

SP: Departamento de Botanica do Estado de São Paulo, Brazil.

Ven: Herbario del Museo Comercial de Venezuela, Caracas, Venezuela.

The majority of collections from the Museo Goeldi are without collectors' numbers. Thus, it has been found necessary to cite them under the numbers that were assigned to them at that institution. The abbreviation "H.A.M.P." stands for Herbarium Amazonicum Musei Paraensis.

CHONDODENDRON Ruíz & Pavon

2. CHONDODENDRON PLATIPHYLLUM (A. St. Hil.) Miers. *Additional specimens examined:* BRAZIL—RIO DE JANEIRO: *Glaziou 9348* (PG). SÃO PAULO: *Dept. Bot. São Paulo 12985* (SP).

This is the first record of the species from the State of São Paulo.

5. CHONDODENDRON LIMACIIFOLIUM (Diels) Moldenke. *Additional specimens examined:* BRAZIL—PARÁ: *Sigueira s.n.* (*H.A.M.P. 8266*) (PG—isotype); *collector undesignated s.n.* (*H.A.M.P. 9565*) (PG), *s.n.* (*H.A.M.P. 10178*) (PG).

The common name "abuta" is recorded for specimens from Pará. In our previous paper (1: 22) we stated that fruits of only four species of *Chondodendron* (namely *C. tomentosum, C. toxicoferum, C. tomentocarpum* and *C. platiphyllum*) were available to us for examination. The above cited specimen (*H.A.M.P. 10178*) of *C. limaciifolium* is in fruit, which is here described for the first time. It is now evident that the fruits of this species are larger than those of *Chondodendron toxicoferum* (*Krukoff 4754* and *H.A.M.P. 4286*), which are about 2 cm. long and 1 cm. wide.

Infructescence racemiform; fruiting peduncles and rachis stout, densely incanous-tomentellous, often greatly abbreviated; fruiting pedicels stout,

1–2.5 cm. long, densely incanous-tomentellous; torus greatly expanded and club-shaped in fruit, 4–5 mm. in diameter; fruit elliptic, symmetric, basally attached, 2.5–2.8 cm. long, 2.1–2.4 cm. wide, rounded at both ends, the exocarp densely flavescent- or sordid-tomentellous, the mesocarp thin, rather woody, the endocarp thin and roughened.

7. CHONDODENDRON TOXICOFERUM (Wedd.) Krukoff & Moldenke. *Additional specimens examined:* BRAZIL: basin of Rio Jurua, *Ule 5631* (type coll. of *C. polyanthum*) (PG). AMAZONAS: basin of Rio Iça, *Ducke s.n.* (*H.A.M.P. 7657*) (PG). ACRE TERRITORY: basin of Rio Acre, *Huber s.n.* (*H.A.M.P. 4286*) (PG).

SCIADOTENIA Miers

1. SCIADOTENIA SAGOTIANA (Eichl.) Diels. *Additional specimens examined:* BRAZIL—PARÁ: *Goeldi s.n.* (*H.A.M.P. 3239*) (PG); *dos Santos s.n.* (*H.A.M.P. 7290*) (PG).

The common name "abuta miry" is recorded for the specimen collected by dos Santos. The species has been known hitherto only from the type collection from French Guiana. The fruits are here described for the first time.

Infructescence dichotomously branched, the branches usually very short; fruiting-peduncles slender, 1.3–2 cm. long, densely cinereous-tomentellous; fruiting pedicels about 3 mm. long, incrassate, densely cinereous-tomentellous, the torus not expanded; fruit obovate, symmetric, basally attached, 1–1.5 mm. long, 7–9 mm. wide, rounded at apex, narrowed to the base, the exocarp very densely puberulent, wrinkled, the mesocarp very thin and probably fleshy when fresh, the endocarp papery, glabrous, very shiny.

2. SCIADOTENIA PARAËNSIS (Eichl.) Diels. *Additional specimen examined:* BRAZIL—AMAZONAS: basin of Rio Jamunda, *Ducke s.n.* (*H.A.M.P. 15685*) (PG).

This is the first record of the species from the State of Amazonas.

8. SCIADOTENIA AMAZONICA Eichl. *Additional specimen examined:* PERU—LORETO: near Iquitos, *Ducke s.n.* (*H.A.M.P. 7588*) (PG).

This is the second collection of the species and the first record of it from Peru. The specimen is the first one known with staminate inflorescences. A description of the flowers follows: staminate flowers: sepals 18, closely imbricate, varying in size, arranged in sets of 3, the outermost set smallest, lanceolate, about 1.2 mm. long and 0.4 mm. wide, gradually attenuate to the apex, the innermost set largest, elliptic, about 2.5 mm. long and 0.8 mm. wide, acuminate at the apex, all densely villous on the back and margins; petals 6, subequal, oblanceolate, about 1.6 mm. long and 0.5 mm. wide above the middle, thin-textured along the margins, thickened in a median ridge, acute at apex, slightly concave, densely villous on the back and margins; stamens 6, free, three longer and stouter than the other three, the glabrous filaments conspicuously ampliate at the apex.

9. SCIADOTENIA CAYENNENSIS Benth. *Additional specimens examined:* BRAZIL—PARÁ: *Huber s.n.* (*H.A.M.P. 519*) (PG); basin of Rio Trombetas, *Ducke s.n.* (*H.A.M.P. 15886*) (PG).

Ducke's specimen is the first record of the species from the basin of Rio Trombetas. He records on the label: "Large vine. Fruits are orange in color."

10. SCIADOTENIA BRACHYPODA Diels. *Additional specimens examined:* BRAZIL—AMAZONAS: basin of Rio Purus, *Goeldi s.n.* (*H.A.M.P. 3934*) (PG—type); basin of Rio Iça, *Ducke s.n.* (*H.A.M.P. 7694*) (PG).

12. SCIADOTENIA SPRUCEI Diels. *Additional specimens examined:* BRAZIL—PARÁ: *Miles Moss 90* (W); basin of Rio Tapajoz, *Ducke s.n.* (*H.A.M.P. 16761*) (PG).

The species has been known hitherto only from two collections from the basin of Rio Negro, State of Amazonas. Ducke's specimen is remarkable for its comparatively large leaves resembling in shape those of *S. brachypoda.* The collector states: "flowers yellowish."

ANOMOSPERMUM Miers

1. ANOMOSPERMUM SCHOMBURGKII Miers. *Additional specimens examined:* BRAZIL—ACRE TERRITORY: Rio Branco, *Ule 7702* (*H.A.M.P. 12809*) (PG). PARÁ: Collares, *Ducke s.n.* (*H.A.M.P. 12616*) (PG). CEARÁ: *Ducke s.n.* (*H.A.M.P. 1503*) (PG); *Huber s.n.* (*H.A.M.P. 1862*) (PG).

3. ANOMOSPERMUM RETICULATUM (Mart.) Eichl. *Additional specimens examined:* BRAZIL—AMAZONAS: basin of Rio Iça, *Ducke s.n.* (*H.A.M.P. 7716*) (PG). PARÁ: basin of Rio Xingu, *Bach s.n.* (*H.A.M.P. 4149*) (PG).

The Bach specimen is the first record of the species from the State of Pará. The fruits are here described for the first time. Fruiting pedicels stout, about 1 cm. long, short-pubescent; torus club-shaped in fruit, conspicuously expanded to 4 mm. in diameter; fruit asymmetric, ovate, basally attached, about 3 cm. long and 2 cm. wide, the exocarp more or less pustulate-roughened except at the smooth and shiny points of contact, the mesocarp very thick, hard, and bony.

6. ANOMOSPERMUM CHLORANTHUM Diels. *Additional specimen examined:* BRAZIL—ACRE TERRITORY: basin of Rio Acre, *Ule 9388* (PG—isotype).

TELITOXICUM Moldenke

1. TELITOXICUM KRUKOVII Moldenke. *Additional specimen examined:* BRAZIL—PARÁ: basin of Rio Curua, *LeCointe s.n.* (*H.A.M.P. 17301*) (PG).

The common name "cipo lingua" is recorded by LeCointe. The species has been known hitherto only from the type collection from the basin of Rio Madeira, State of Amazonas.

3. TELITOXICUM INOPINATUM Moldenke. *Additional specimen examined:* BRAZIL—PARÁ: near Almeirim, *Ducke s.n.* (*H.A.M.P. 17261*) (PG).

This is the first record of the species from Brazil.

5. TELITOXICUM DUCKEI (Diels) Moldenke. *Additional specimen examined:* BRAZIL—PARÁ: basin of Rio Mapuera, *Ducke s.n.* (*H.A.M.P. 9012*) (PG—isotype).

6. TELITOXICUM MINUTIFLORUM (Diels) Moldenke. *Additional specimen examined:* BRAZIL—AMAZONAS: basin of Rio Negro, *Froes 11998* (N).

ABUTA Barrère

2. ABUTA OBOVATA Diels. *Additional specimen examined:* BRAZIL—AMAZONAS: basin of Rio Negro, *Froes 11997* (N).

This is the first record of the species from Brazil.

5. Abuta velutina Gleason. *Additional specimen examined:* Brazil—amazonas: basin of Rio Negro, *Froes 12001* (N).

This is the first record of the species from Brazil. It has been known hitherto only from the type collection from Venezuela.

8. Abuta imene (Mart.) Eichl. *Additional specimen examined:* Brazil—pará: basin of Rio Jamunda, *Ducke s.n.* (*H.A.M.P. 11723*) (PG).

This is the first record of the species from the State of Pará.

9. Abuta colombiana Moldenke. *Additional specimen examined:* Brazil—amazonas: basin of Rio Iça, *Ducke s.n.* (*H.A.M.P. 7708*) (PG).

This specimen is placed here tentatively. Its inflorescences are pistillate and only staminate inflorescences have been seen hitherto of this species. Neither are pistillate inflorescences available of the obviously related *A. grandifolia,* so that comparisons cannot be made. The foliar characters of our specimen, however, match so perfectly those of *A. colombiana* and so poorly those of *A. grandifolia,* that it seems most probable that it represents the first Brazilian collection of the former species. A brief description of the flowers is given herewith: pistillate flowers: sepals 6, the outer 3 small, lanceolate, about 0.7 mm. long and 0.3 mm. wide, attenuate from the base to the acute apex, minutely puberulent on the back, the inner 3 much larger, broadly ovate or subrotund, about 1.5 mm. long and wide, rounded to a very short subacute point at the apex, rounded or subcordate at base, densely short-puberulent on the back; carpels 3, densely appressed-pubescent.

11. Abuta grandifolia (Mart.) Sandw. *Additional specimens examined:* Venezuela—bolívar: *Ll. Williams 11437* (Ven). Brazil—matto grosso: *Dep. Bot. São Paulo 8185* (SP). acre territory: basin of Rio Acre, *Ule 9386* (PG), *9387* (PG). amazonas: basin of Rio Purus, *Huber s.n.* (*H.A.M.P. 1414*) (PG), *s.n.* (*H.A.M.P. 4639*) (PG); basin of Rio Iça, *Ducke s.n.* (*H.A.M.P. 7641*), *s.n.* (*H.A.M.P. 7689*) (PG); basin of Rio Negro, *Krukoff 12104* (N); *Froes 11999* (N). pará: basin of Rio Tapajoz, *Ducke s.n.* (*H.A.M.P. 15817*) (PG); basin of Rio Jamauchim, *Snethlage s.n.* (*H.A.M.P. 10105*) (PG); basin of Rio Ariramba, *Ducke s.n.* (*H.A.M.P. 11319*) (PG), *s.n.* (*H.A.M.P. 8056*) (PG); Bragança, *collector undesignated s.n.* (*H.A.M.P. 9808*) (PG); Oriximina, *Ducke s.n.* (*H.A.M.P. 7868*) (PG); Murutucú, *Rodriguez s.n.* (*H.A.M.P. 2820*) (PG); Almerim, *Ducke s.n.* (*H.A.M.P. 3060*) (PG), *s.n.* (*H.A.M.P. 3454*) (PG).

13. Abuta Selloana Eichl. *Additional specimens examined:* Brazil—são paulo: *Dep. Bot. São Paulo 946* (N, SP), *12986* (SP), *12988* (N, SP), *19627* (SP), *28429* (N. SP). matto grosso: *Dep. Bot. São Paulo 8184* (SP).

This is the first record of the species from the State of Matto Grosso. The common name "quina brava" is recorded for specimens from the State of São Paulo.

14. Abuta rufescens Aubl. *Additional specimens examined:* Brazil—amazonas: basin of Rio Negro, *Krukoff 12105* (N); *Froes 12000* (N).

The above specimens are the first record of the species from the basin of Rio Negro.

16. Abuta Grisebachii Triana & Planch. *Additional specimen examined:* Brazil—amazonas: basin of Rio Negro, *Froes 12002* (N).

17. ABUTA CANDOLLEI Triana & Planch. *Additional specimen examined:* BRAZIL—PARÁ: near Obidos, *Ducke s.n.* (*H.A.M.P. 2897*) (PG).

This specimen exhibits staminate flowers whose inner petals are considerably larger than in any previously examined and cited material. The inner petals here are broadly elliptic, about 2.4 mm. long and 1.7 mm. wide.

19. **Abuta brevifolia** Krukoff & Moldenke, sp. nov. Frutex scandens: ramulis gracilibus glabris vel leviter cinereo-pulverulentis; petiolis 7–15 mm. longis, ad apicem valde incrassatis; laminis foliorum coriaceis ellipticis 3.3–7 cm. longis, 2.3–5 cm. latis, ad apicem acutis vel brevissime acuminatis vel obtusis, ad basin rotundatis vel subacutis, subrevolutis, supra subglabris nitidis, subtus obscure pulverulento-puberulis vel subglabris nitidis, 3-plinerviis; infructescentiis axillaribus vel supra-axillaribus racemiformibus foliosis geminatis leviter puberulis.

Description: A woody vine; branchlets slender, glabrous or lightly cinereous-pulverulent; petioles 7–15 mm. long, conspicuously incrassate at apex, lightly puberulent; leaf-blades coriaceous, elliptic, 3.3–7 cm. long, 2.3–5 cm. wide, acute or very shortly acuminate at apex, varying to obtuse, entire and somewhat revolute along the margins, rounded or subacute at base, subglabrous and shiny above, obscurely pulverulent-puberulent or subglabrous and shiny beneath, 3-plinerved; midrib and primary veins very slightly subimpressed above, sharply prominent beneath, the primaries issuing a few mm. above the base of the blade; secondaries numerous, issuing rather irregularly at right angles to the primaries, subparallel to each other, prominulous above, more sharply so beneath; veinlet reticulation rather abundant, obscure above, rather distinct under a hand-lens beneath; inflorescence not seen; infructescence axillary or supra-axillary, racemiform, leafy, borne in pairs, far surpassing the subtending leaves; fruiting peduncles rather slender, 3.5–4 cm. long, lightly puberulent; rachis similar to the peduncles in all respects, 11–12 cm. long, bearing several rather distant leaves and fruits; fruiting pedicels slender, 6–10 mm. long, appressed-puberulent; torus greatly expanded and club-shaped in fruit, 3–3.5 mm. in diameter; fruit oblong, asymmetric, sublaterally attached, about 2 cm. long and 1.3 cm. wide, the exocarp lightly pulverulent and venose, the mesocarp rather thick, very hard and bony, the endocarp thin, stramineous and shiny.

The type specimen has its branchlets, petioles, and infructescences more or less densely covered with a cinereous and closely appressed growth of a species of mold, imparting to the plant a very conspicuous but deceptive ashy-gray appearance. The collection was cited by Diels (4: 197) in remarks under *A. guyanensis* Eichl. [= *A. grandifolia* (Mart.) Sandw.].

Specimen examined: BRAZIL—PARÁ: basin of Rio Mapuera, *Ducke s.n.* (*H.A.M.P. 8976*) (PG—TYPE).

ELISSARRHENA Miers

1. ELISSARRHENA GRANDIFOLIA (Eichl.) Diels. *Additional specimens examined:* BRAZIL—ACRE TERRITORY: basin of Rio Jurua, *Ule 5526* (type coll. of *Anomospermum Ulei*) (PG); basin of Rio Purus, *Ule 9389* (PG).

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The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Papers that relate exclusively to bacteriology, forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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(exclusive of fungi)

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BULLETIN OF THE TORREY BOTANICAL CLUB
69(3): 167–183 MARCH, 1942

INTERMITTENT GROWTH OF FRUITS OF PHALAENOPSIS. A CORRELATION OF THE GROWTH PHASES OF AN ORCHID FRUIT WITH INTERNAL DEVELOPMENT

ROBERT E. DUNCAN AND JOHN T. CURTIS[1]

It has been recognized that the orchid ovary is not mature at the time of pollination since the work of Brogniart (1831) and confirmation of his findings by a number of mid-nineteenth century botanists. Hildebrand (1863), Treub (1883), and Guignard (1886) determined the periods of time which elapse between pollination, maturation of the macrogametophytes, fertilization, and appearance of the embryos, respectively, for a considerable number of species. Veitch (1888) extended this type of observation to *Cattleya labiata* var. *mossiae*. In general, the periods of time between the phases of the reproductive process vary greatly from genus to genus and from species to species within the same genus. For example, Hildebrand (1863) found that 110 days after pollination the ovaries of *Dendrobium nobile* contained ovules possessing immature macrogametophytes and both integuments, and that 130 days after pollination embryos were developing. In *Orchis mascula* the respective periods were 19 and 28 days; in *O. pyramidalis* 7 and 10 days.

Brown (1833) commented that the first pollen effect is the enlargement of the ovary and ovules. Crüger (1851) observed that pollination had a dynamic effect on fruit development in *Vanda* in that ovules are only formed after pollination. This effect, he believed, took place before the entrancé of the pollen tubes into the ovary cavity, in contrast to the effect brought about by the material contribution of the pollen tubes at the time of fertilization. Hildebrand (1863) clearly recognized the "double effect" of pollination which he said was, first, the increase in size of the ovary and the maturation of the ovules, and second, fertilization. He recognized that unpollinated flowers fell off the plant, even though the ovules in some cases grew to a limited extent. Hildebrand (1865) found that in many cases foreign pollen induced fruit-set. He considered that a green enlarging ovary was of primary importance to the development of the ovules.

The work of Fitting (1909) and Laibach and Maschmann (1933) demonstrated by the behavior of the column that the pollinia contain a growth-regulatory substance. In general they studied an immediate pollination reaction characterized by the growth of the edges of the stigmatic cavity in

[1] Part of the cost of illustrations for this paper was borne by the Lucien M. Underwood Memorial Fund.

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such a fashion as to enclose the pollinia.[2] Guignard (1886) suggested that the enlargement of the ovary accompanied the growth of the pollen tubes to the base of the ovary.

That pollination is necessary for fruit-set and in some indirect manner for ovule development is clearly demonstrated by Hurst's (1898) report of matroclinous progeny resulting from attempted crosses between *Zygopetalum mackayi* and various species of *Odontoglossum, Oncidium,* and *Lycaste,* respectively. Suessenguth (1923) has shown that embryos formed in such crosses are of apomictic origin. They are not formed, however, unless pollination takes place.

Phalaenopsis, the moth orchid, a Malasian member of the *Sarcanthinae,* grows as an epiphyte in the rain forests. Like other orchids the fruit is a three-valved capsule containing innumerable minute seeds. In the United States and Europe it is frequently used as a source of cut flowers, for under good growing conditions a plant with 10–12 basal leaves may produce a laterally branched inflorescence with as many as 170 flowers. The genus has seldom been used as a subject of cytological, morphological, or physiological research. Palm (1920) reported that cytokinesis is simultaneous after meiosis in pollen mother cells. Treub (1879) recorded the presence of haustorium-like suspensors in the embryos of *Phalaenopsis grandiflora, P. schilleriana,* and *P. spectabile.* In a series of papers beginning with that of Fitting (1909) and culminating with that of Laibach and Maschmann (1933) *Phalaenopsis* was one of the orchids in whose pollinia a growth regulatory substance was demonstrated and studied.

Burgeff (1934), using seeds of 11 species and hybrids of *Phalaenopsis,* reported that the great majority of them gave germination percentages of less than 10 per cent when grown on "vitamin-free" media, while the addition of an unidentified vitamin or growth substance isolated from yeast and certain soil fungi increased the germination to 90 or 100 per cent. On the other hand seeds of *Phalaenopsis sanderiana* and *P. equestris* germinated 100 per cent without an external source of growth substance.

METHODS AND MATERIALS

The various individual studies reported herein were made in winter and

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[2] Our attention has been called to a recent paper on the effects of synthetic growth-regulatory compound on the column in bringing about several postfloral phenomena (**Hubert, B., & Maton, J.** 1939. The influence of synthetic growth controlling substances and other chemicals on postfloral phenomena in tropical orchids. Biol. Jaarb. **6**: 244–285). The same authors produced parthenocarpic fruits in a *Cymbidium* hybrid and in *Oncidium longipes* by the application of pure crystals of naphthaleneacetic acid on the stigmas (Parthenocarpie en groeistof. Natuurwetenschap. Tijdschr. **21**: 339–348. 1940). These fruits reached a considerable size. Unfortunately we have never seen the original papers and have only the information derived from abstracts.

early spring of subsequent years in the botany greenhouses of the University of Wisconsin and the conservatory of Dr. C. K. Schubert, both in Madison, Wisconsin, and in the greenhouses of the Morris Arboretum and the Botany Department of the University of Pennsylvania, both in Philadelphia.

In these studies plants of *Phalaenopsis pamala, P. fontainebleau, P. aphrodite,* and *P. schilleriana* were used. Of these the latter two are naturally occurring forms; *P. fontainebleau* is a hybrid between *P. wiganiae* (a primary hybrid between the species *P. schilleriana* and *P. stuartiana*) and *P. schilleriana. Phalaenopsis pamala* is a hybrid between *P. fontainebleau* and *P. elizabethiae* (a primary hybrid between the species *P. amabilis* and *P. rimstadtiana*).

In any one study the amount of pollen applied to an individual stigmatic surface was macroscopically similar. It consisted of one or two pollinia, the latter being the probable number in natural pollination since each pair of pollinia derived from an anther is connected to an adhesive disc. The pollinations were self, close, or reciprocal. Flowers of approximately the same age, on various branches of the same inflorescence, were pollinated or used as pollen source in close pollinations. In reciprocal pollination the particular pollinium or pair of pollinia applied to any one flower was selected at random from the lot of pollinia derived from the flowers to be pollinated on the other plant. Of the total number to be used for study an approximately equal number of flowers on each branch of an inflorescence was pollinated.

The diameter of the developing fruit was measured to 0.1 mm. with vernier calipers; the length with vernier calipers or a flexible millimeter rule. These measurements were made possible, although the ovary is inferior, by the fact that immediately below the level of sepal insertion the fused structure, representing ovary and receptacle, is lined with six grooves which terminate uniformly in the flower and early stages of ovary maturation. Freehand sections show that the base of the ovary cavity is closely associated with the groove ends. As the development of the ovary proceeds the groove lengths of an individual ovary may vary slightly from the bottom to the top sides, the top grooves generally being shorter. This difficulty was met in different studies either by taking an average of the length of top and bottom grooves at each measurement, beginning at the time when the discrepancy appeared, or by disregarding all but one groove which then became the index of ovary growth in length. In occasional flowers the association of groove ends with the termination of the ovary swelling is varied by the grooves stopping slightly short of the swelling or passing beyond it down the pedicel. These variations may be noted on different ovaries of the same plant. Such phenomena were disregarded and the groove measurements were used. Since the ovary tapers from the level of perianth insertion

to the pedicel during the earlier stages of development, a slight distance above or below the approximate middle will give larger or smaller diameters respectively. Small cuticular scars, where the last measurement was made, gave reasonable certainty that the diameter measurements were taken at the same level. In later studies, ovaries were marked with India ink at the approximate center and at both proximal and distal ends. In this way measurements at the same level were assured each time.

In preliminary studies a number of flowers were pollinated and, at set periods of time, one of the ovaries was measured. The ovary concerned was removed, weighed, and fixed in Randolph's modification of Nawaschin's fixing solution for use in determining the state of ovule development and the fruit wall changes. Entering the measurements obtained from subsequent fruits and joining the points of length and of diameter respectively resulted in two growth curves. Such curves are open to criticism on the permissibility of connecting points so derived. Such a study, however, was necessary to locate the various internal changes with respect to growth phases.

In a second series of studies a number of flowers on a single plant were pollinated. The group was sampled by removing at random a fruit for determination of the state of internal development and for weighing. The points of the growth curves for length and diameter were determined by the average of a smaller and smaller number of developing ovaries as the study progressed. A growth curve erected on such data can be criticized on the basis that the larger share of food, available to the remaining young fruits after removal of one, might falsify the slope of the curve immediately following the removal.

In a third series of studies all the fruits were allowed to develop to maturity. While these studies did not disclose fruit wall or ovular changes they provide a series of continuous measurements of individual fruits free from the effect of shifting food supply except as may occur naturally. (The illustrations present graphically the development of representative fruits from this series of studies.)

After the fruits had ripened, examinations were made to determine the percentage of seeds containing fully formed embryos. The seeds were usually sharply divided into two groups: those containing apparently normal embryos and those consisting only of empty seed coats. As a further test, samples of seeds from each of the pods were sown on a medium previously found suitable for the germination of a variety of orchid seeds (Curtis 1936), modified by addition of the necessary complement of vitamins. These cultures were maintained under constant environmental conditions (temperature, 30° C.; relative humidity, 80 per cent; light, 20 hours per day at

150 f. c.). Germination is reported in the legend of the graphs as percentage of seeds which originally contained embryos, disregarding those which were empty.

RESULTS

Within 24 hours after pollination the petals and sepals are noticeably wilted; within 48 hours the turgidity is lowered to the extent that the venation of petals and sepals is distinct. At the close of this period the petals have folded forward over the column and the upper sepal has bent forward from its former reflexed position. These perianth changes are concomitant with growth of the column in width and thickness and with growth of the edges of the stigma over the cavity in which the pollinia become imprisoned. The columnar growth continues for 9 or 10 days, the major part taking place during the first few days. The column remains swollen during much of the early period of fruit maturation; later it dries down considerably.

The first response of the ovary to pollination is a slight increase in its curvature immediately below the insertion of the perianth. This curvature is soon lost in the general growth of ovary in length and diameter. The accompanying graphs (figs. 1, 2, 3) illustrate the extent and general rate of growth and the fact that the curves for growth in both diameter and length are not simple sigmoid ones. Comparison of the growth curves of *Phalaenopsis fontainebleau* (fig. 1), *P. schilleriana* (fig. 2), and *P. pamala* (fig. 3) shows the same general configuration to be present although there are minor differences in the total extent and the time and duration of the maximum rate of growth. Figure 4 shows the relationship which the increase in fresh weight of the ovary of *P. fontainebleau* bears to the increase in calculated volume of the ovary.

Cross sections of the ovary of the unpollinated flower show the wall, slightly triangular in outline, to be made up of six lobes each with a vascular supply. Three lobes occupy the corners of the triangle. The three lying between each pair of these bear the placentae, long ridges inserted opposite the vascular supply throughout the length of the ovary. In cross section the placentae appear somewhat anchor-shaped and closely fit together, leaving little space in the central part of the ovary.

Within two days after pollination nuclear and cell divisions can be found in cells of the inner faces of the three sterile lobes and in the cells constituting the margin of the placentae. The cell divisions in the placentae have no particular orientation, the result being a general increase in the shoulders. This building up of the flanges or shoulders leads to an inversion of the placental shape from a ridge with reflexed margins to a troughed structure with flanges jutting above the attached median portion. The fact that the two flanges may reach a greater length than the attached portion eventu-

FIG. 1

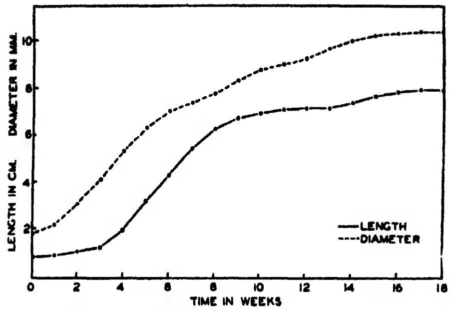


FIG. 2

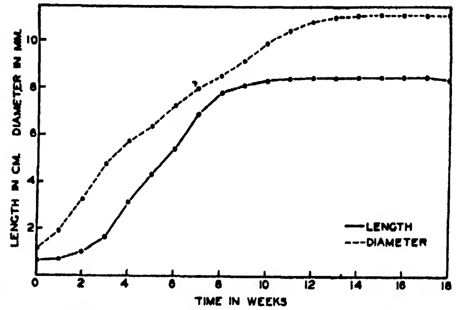


FIG 3

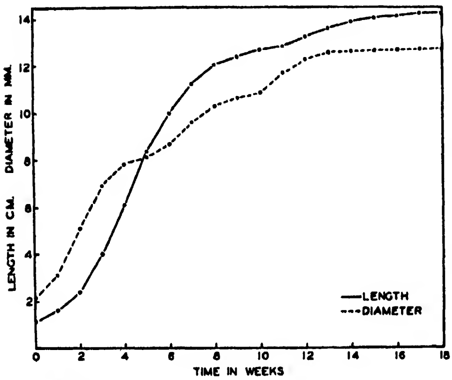


FIG. 4

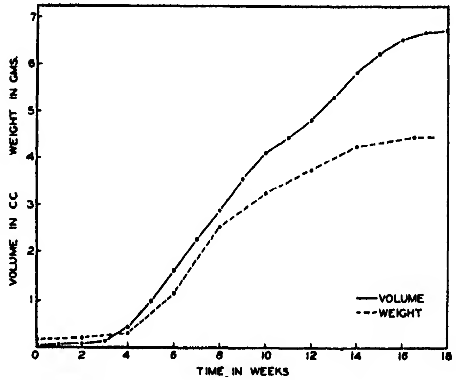


FIG 5

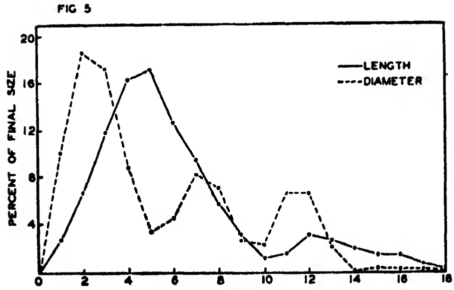


FIG 6

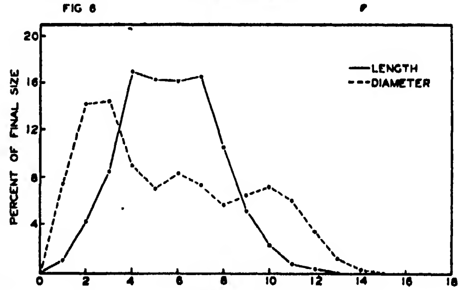


FIG. 7

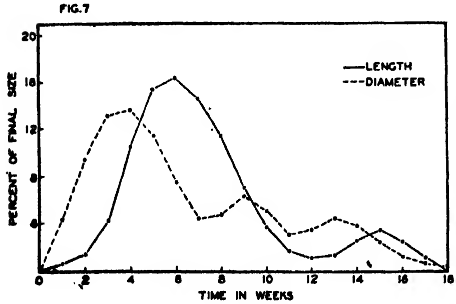
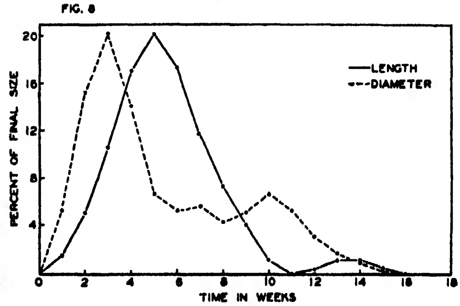


FIG. 8



ally leads to undulations of the flanges. The flanges are not necessarily continuous along the length of the placentae but may consist of a number of flat plate-like structures.

Nuclear and cell divisions in the cells of the ovary wall layers toward the cavity are mainly periclinal, although a few take place along the longitudinal axis of the ovary. The periclinal division and subsequent periclinal growth of the three or four inner cell rows and the enlargement of the outer cell layers allow an increase in the ovary cavity. While it seems probable that there would be a number of nuclear and cell divisions taking place along the longitudinal axis to provide for growth in length of the ovary, aside from the divisions just mentioned and another series involving the epidermal and hypodermal cell layers about 2 or 3 weeks after pollination, no other areas in which cell divisions were taking place have been noted. The cells making up the ovary wall of the unpollinated flower are only slightly elongated, the cells of the inner cell layers being less elongated than those of the outer. The growth and maturation of cells already present in the ovary wall layers account for the growth in length of the ovary and to a limited extent for the growth in diameter.

The pollen germinates within the first four days after being placed on the stigma. The tubes grow down the canal of the column until they reach the ovary cavity, where the single group of tubes divides into three strands, one passing to each of the three placentae. Upon reaching the placenta a strand of pollen tubes divides again; each half passes down one side of the placenta. Fourteen days after pollination the longest pollen tubes have reached the base of the ovary cavity, but the majority are still growing in the upper part of the ovary. Two nuclei are present near the tip of the pollen tube. While the pollen tubes are growing down along the placentae, rows of blunt-tipped glistening hairs arise from the inner ridge of the three sterile lobes of the ovary. These hairs increase in length and persist until the fruit is mature. Occasionally in certain crosses the pollen does not germinate or, if it does, the pollen tubes do not penetrate to the ovary cavity.

Explanation of figures 1–8

FIGS. 1–3. Growth curves of individual fruits of *Phalaenopsis*. FIG. 1. *P. fontainebleau* at the Morris Arboretum. FIG. 2. *P. schilleriana* at Dr. C. K. Schubert's conservatory. FIG. 3. *P. pamala* at the University of Wisconsin. FIG. 4. Curves presenting the increase in calculated volume and fresh weight, respectively, of fruits of *P. fontainebleau* at the Morris Arboretum. FIGS. 5–8. Curves presenting the weekly growth increments of individual fruits of *Phalaenopsis*. FIG. 5. *P. pamala* at the University of Wisconsin. (91.0 per cent seeds possessing embryos; 92.6 per cent germination.) FIG. 6. *P. schilleriana* at Dr. C. K. Schubert's conservatory. (93.8 per cent possessing embryos; 95.1 per cent germination.) FIG. 7. *P. fontainebleau* at the Morris Arboretum. (88.5 per cent seeds possessing embryos.) FIG. 8. *P. fontainebleau* at Dr. C. K. Schubert's conservatory. (98.0 per cent seeds possessing embryos; 96.2 per cent germination.)

In *Phalaenopsis pamala* at the botany greenhouses of the University of Wisconsin the placentae proliferate for 3 or 4 weeks; in *P. schilleriana* at the Morris Arboretum this period lasts over 6 weeks. The final branches in either case are small mound-like structures which differentiate during the ensuing 10 to 14 days into an axial row of cells surrounded by a sheath of cells one row thick. The inner integument appears as a collar-like growth at this time and is shortly followed by the initiation of the outer integument. As this latter structure develops the ovule becomes anatropous, the funiculus being fused to one side of the outer integument. By the time the integuments have reached the apex of the nucellus, the terminal cell of the axial row has enlarged to become the macrospore mother cell. In *P. pamala* ovules in this stage of development are present about 5 weeks after pollination or 3 weeks after their initiation; in *P. schilleriana* at the Morris Arboretum the periods are 12 and 6 weeks respectively.

During the course of development of the macrogametophyte the upper part of the ovule elongates and the outer integument grows on beyond the inner so that there is some space between the micropyles of the two integuments. Fertilization takes place in *P. pamala* during the ninth and tenth weeks. Since the ovules are not all in exactly the same stage this period is not sharply defined. With ovules containing two and three-celled embryos may be found a few ovules, at the placental extremities, which are still in the macrospore mother cell stages. Capsules of the *P. schilleriana* series at the Morris Arboretum opened in the fifteenth week without any viable seeds. Two weeks earlier the ovules had become anatropous, the two integuments just growing over the apex of the nucellus.

Shortly after fertilization the pollen tubes disappear. The ovule as a whole elongates markedly. The embryo grows for a period of several weeks and comes to displace all the tissues of the ovule except the outer integument which becomes the sole layer of the seed coat. The embryo of the mature seed fills only a portion of the cavity within the seed coat. Suspensors in *P. pamala* were not observed to grow out through the micropyle into the placenta. The funiculus has no vascular supply.

At the close of the period of fruit maturation the changes are slow. The grooves become deeper; the dark green color fades to gray green; lenticels become evident; and there is a decided increase in the amount of cutin. About six months after pollination the fruits of *P. pamala* become slightly softer and yellowish.

Study of ovary wall changes shows that, while there is a period of accelerated growth followed by a period of slower growth for both placental depth and wall thickness, the two structures come to occupy a smaller percentage of the diameter of the entire fruit. The ovary cavity increased rapidly during three periods of ovary development. This increase in ovary

cavity is illustrated by figure 4 which shows a divergence between the weight and volume of the fruit, this divergence becoming particularly noticeable after the eighth week. *Phalaenopsis pamala*, *P. schilleriana*, and *P. fontainebleau* all in the conservatory of Dr. Schubert, and *P. fontainebleau* in the botany greenhouses of the University of Pennsylvania each had approximately the same periodicity of growth as *P. pamala* in the Wisconsin botany greenhouses.

Table 1 summarizes the morphological changes and presents a general schedule of fruit development.

TABLE 1

Schedule of events in fruit development

Period of time after pollination	Morphological and physiological changes
2 days	Perianth wilted; edges of stigmatic cavity enclose pollinia; nuclear and cell divisions in cells of inner parietal layers of ovary wall and beginning of such divisions in placentae.
4 days	Pollen germinates.
10 days	Columnar growth about finished.
25 days	Placental proliferation decelerating.
35–40 days	Differentiation of ovules.
55–60 days	Maturation of macrogametophytes.
63–70 days	Fertilization; degeneration of pollen tubes.
180–190 days	Fruit matures.

DISCUSSION

Inspection of growth curves for both length and diameter shows that each is made up of several sigmoid parts. Plotting weekly increments of growth against time results in a graph which clearly portrays three phases of growth in diameter and one, sometimes two, in length (figs. 5, 6, 7, 8). The increment of growth for each week is plotted in terms of percentage of the final size reached by that particular fruit. In this way, all graphs are on the same scale and are directly comparable with one another. The plotted increment points are connected by lines for ease in inspection. In *Phalaenopsis pamala* (fig. 5) the first and larger phase of growth in length falls between the maxima of the first two growth phases in diameter at about the time of the minimum rate between the two. In *P. schilleriana* (fig. 6) the first phase of growth in length is bimodal except under unusual conditions, possessing two maxima of approximately equal magnitude. The first of the two maxima has about the same relationship to growth in diameter as does the peak rate of the first phase of growth in length in *P. pamala*. In *P. pamala* (fig. 5) after growth in length has reached its maximum rate and is becoming slower there is a period during the sixth and seventh week of development when the rate of deceleration is halted. This corresponds roughly

to the second maximum in the first phase of growth in length in *P. schilleriana* (fig. 6). In both forms this main growth phase lasts about 10 weeks.

In all the forms studied there are at least three periods of accelerated growth in diameter (figs. 5, 6, 7, 8). When there is a second phase of elongation it is of comparatively small magnitude and is loosely associated with the last phase of accelerated growth in diameter. The fact that data from cross pollination between different plants of the same hybrid (fig. 5), between different hybrids, and between hybrids and species (figs. 6, 7), as well as self and close pollinations of hybrids or species, all yielded growth curves of the same fundamental type emphasizes that this type of growth is the rule, at least in this section of the genus *Phalaenopsis,* and that there must be common morphological and physiological behaviors. The possibility that the type of growth found is a response to chance environmental factors is ruled out by this common behavior and by the fact that studies of all forms except *P. aphrodite* were made under several greenhouse conditions in the same or different years and started at various times of the year. For example, nine studies were made with *P. pamala* over a period of four years in two different greenhouses and with pollination dates varying from October to March, yet all growth curves are essentially alike. (This does not imply that environment will not affect the magnitude or the rate of the phases of ovary development; a comparison of figures 7 and 8 does not bear out such a conclusion.) The duration of any one experiment (the time required for fruit ripening) was approximately six months. The high percentage of seeds set and of seeds which germinated demonstrate that the fruits studied were not abnormal.

In *Phalaenopsis,* as has been pointed out, the first noticeable changes after pollination are wilting of the perianth segments, enclosing of the pollinia by the stigma, an increase of the curvature of the ovary, and lengthening and swelling of the ovary. Within two days the cells forming the ovary wall layers next the central cavity are dividing. This is long before the pollen tubes enter the ovary; actually these changes take place whether or not the pollen germinates. In case the pollen fails to germinate or the pollen tubes fail to enter the ovary cavity, the ovary drops off the inflorescence during the ensuing two weeks by means of an abscission layer at the base of the pedicel. Thus the presence of the pollinia on the stigma is sufficient to produce the first three effects and to initiate ovary growth. All these changes are probably in response to growth-regulatory substances present in the pollinia. The perianth and column changes were the particular object of study by Fitting and his coworkers. The wilting of the perianth takes place at the time of the initiation of column and ovary growth when there is a decided modification of water relations. The initial dose of growth-

regulatory substance derived from the pollinia is insufficient, however, to prevent fruit drop unless there is some further development.

This further development is undoubtedly the entrance of pollen tubes into the ovary. The increment curves of many individual ovaries show an initial lag in growth in length; at the conclusion of this lag there is a sharp increase in rate. It is at this time that the pollen tubes enter the ovary cavity. Pollen tube growth approximately keeps pace with length growth of the ovary. The pollen tubes in the ovary may function by secreting growth-regulatory compounds, by providing a tactile stimulus, or by exerting a mechanical force by reason of their hydrophilous nature.

The second period of elongation falls at a time when the embryos are growing. Its significance is little understood; certainly when present it would function in stretching the placenta, thus somewhat separating the seeds. It could be caused by growth-regulatory substances produced within the embryo. These substances may pass through the suspensor which, however, has not been observed to grow into the placenta in the forms studied. The funiculus is not vascularized but is the alternative route of translocation. A mechanical response to the collective size increase of the embryos is another possible cause.

As previously pointed out growth in diameter takes place in at least three well defined phases. Within two days after pollination a series of nuclear and cell divisions occurs within the inner wall layers of the ovary cavity. These divisions are mainly periclinal, few longitudinal. Except for another series of divisions occurring within the epidermal and hypodermal layers and scattered divisions in the regions adjoining the vascular bundles, all taking place several weeks later, there is no increase in the number of cells making up the fruit coat. The enlargement of cells already present must cause the major part of the increase in fruit wall thickness.

A study of changes in the fruit wall shows that this increase takes place early and can only partially explain the initial phase of growth in diameter. The increase in the diameter of the ovary cavity, however, has at least three growth phases exactly corresponding to the gross diameter changes. Since the growth of the cells of the inner layers of the ovary is periclinal, the substances in the pollinia and produced by the pollen tubes, or produced by the pollen tubes alone, probably regulate this growth during the first phase of increase in length and diameter respectively. (The dependence of diameter increase upon pollen tubes is borne out by zonal growth of the ovary, the swelling advancing as the strands of pollen tubes grow downward.)

During the first phase of diameter growth placental proliferation eventually leads to the initiation of ovule rudiments. While the rate of diameter growth is decelerating, increasing numbers of these rudiments are found.

At the same time the hairs which serve later as elaters develop from the innermost cell layers of the sterile lobes. Although there is no sharp break from one morphological stage in the reproductive cycle to the next, macrosporogenesis is taking place in the greatest number of ovules while the diameter growth is at or near a minimum rate. The close of the first phase of growth in diameter may rest with disturbed food relations or other changes occasioned by the heavier demands of the macrospore mother cells undergoing reduction division at that time. During the ensuing phase of growth in diameter the macrogametophytes develop; at the close of this development the ovules elongate slightly before fertilization. Fertilization takes place during a second period of minimum rate of growth in diameter. During the next few weeks the ovules increase in both dimensions while the embryos are growing; at this time the ovary undergoes the third phase of diameter increase. The pollen tubes disintegrate and disappear shortly after fertilization. It appears probable that the material derived from the pollen tubes functions either as a stimulus or as an actual energy source for the third increase in diameter of the ovary. The latter interpretation is supported by the known high content of pentosan-like hydrophilic colloids in the active microgametophyte.

It is noteworthy that each of the steps in internal development—(1) placental proliferation and ovule initiation, (2) maturation of the ovule in preparation for fertilization, and (3) seed development (embryo growth)—which demand more space are provided for by three cycles of diameter growth. At the time of macrosporogenesis and at the time of fertilization the rate of diameter growth is slow followed by an increased rate.

At the time of fertilization a group of sterility factors makes its appearance. If for genetical or cytological reasons macrosporogenesis fails, no macrogametes can be formed and fertilization cannot take place, even though the ovules elongate and there is a second phase of growth in diameter. A similar result would be obtained if the pollen tubes failed to reach the ovules (which is most common), if the act of fertilization itself failed, or if the zygotes or young embryos degenerated. (The failure of the *Phalaenopsis schilleriana* series at the Morris Arboretum was caused by some one of this group of factors.) No further growth occurs if fertilization does not take place; it follows that the third phase of diameter growth is a response to the fertilization stimulus. It seems that if fertilization took place and embryos developed to the two- or three-celled state before a degeneration set in that a third growth phase would follow. We have no data on this point.

Since the *Phalaenopsis* fruit approaches a cylinder in shape, computing the volume from length and diameter measurements is feasible. Any error involved, such as the slight tapering of the ovary base, would be more or less uniform. A growth curve erected (volume vs. time) very nearly approaches

—up to the time of embryo development—the logarithmic growth curve. A curve (fig. 4) representing increase in fresh weight follows the volume increase more closely throughout than it does curves representing either growth in length or diameter. The specific gravity decreases as the fruit matures, in consequence of the increased amount of air space within it, the decrease becoming most noticeable as the macrogametophytes mature.

The interpretation as presented is an elaboration and refinement of the ideas expressed by Crüger (1851) when he pointed out the "dynamic" effect of pollination in addition to the necessity of pollination prior to fertilization, and by Hildebrand (1863) when he demonstrated the "twofold" effect of pollination. While Brown (1831), Crüger, Hildebrand, and Guignard (1886) all commented on this dynamic effect of pollination, which is so obvious in the orchids since it precedes fertilization by a long period of time, and while the latter two knew that the ovary was not ready for fertilization at the time of pollination, they made no correlations between internal development and the various growth phases. Table 2, presenting the sequence of

TABLE 2

Summary of fruit changes and their possible causes

Cause	Result	Comment
1. Pollination effect.	1. Perianth wilting. 2. Increase in ovary curvature. 3. Initiation of nuclear and cell divisions in inner parietal layers of ovary. 4. Initiation of growth in both length and diameter.	The growth initiated is closely associated with following phases. Can only be dissociated in incompatible crosses. Its effect can be duplicated by the application of growth regulatory substances to the stigma.
2. Effect of entrance of pollen tubes into ovary.	1. Prevention of fruit drop.	Fruit set placed here because flowers in incompatible crosses, even though pollen germinates, fall off the inflorescence sooner than unpollinated flowers of same age.
	2. Grand phases of growth in both length and diameter.	At time of placental proliferation. Culminates in initiation of ovule rudiments with respect to diameter growth.
3. (Cause not clear)	1. A second phase of growth in diameter.	At time of macrogametophyte maturation. Cannot be dissociated experimentally from preceding growth phase in diameter. Cause may lie in ovary itself.
4. Fertilization effect.	1. A third phase of diameter growth and at times a second in length.	Not present if fertilization fails. A group of sterility factors separates this from preceding growth phases. Seed development at this time.

growth in relation to causality and to morphological changes, is self-explanatory.

Figure 9, a generalized curve of the increments of growth in diameter, shows a possible analysis into three component curves. Another component, the growth caused by the presence of pollen alone, which would occur at the beginning, is not separated from the first phase of growth because there is no method of judging from incompatible crosses the total extent of this effect. The general appearance of each segment is that of a normal distribution curve and may be so considered for the rate of the process concerned (ovule rudiment formation, macrogametophyte maturation, or embryo develop-

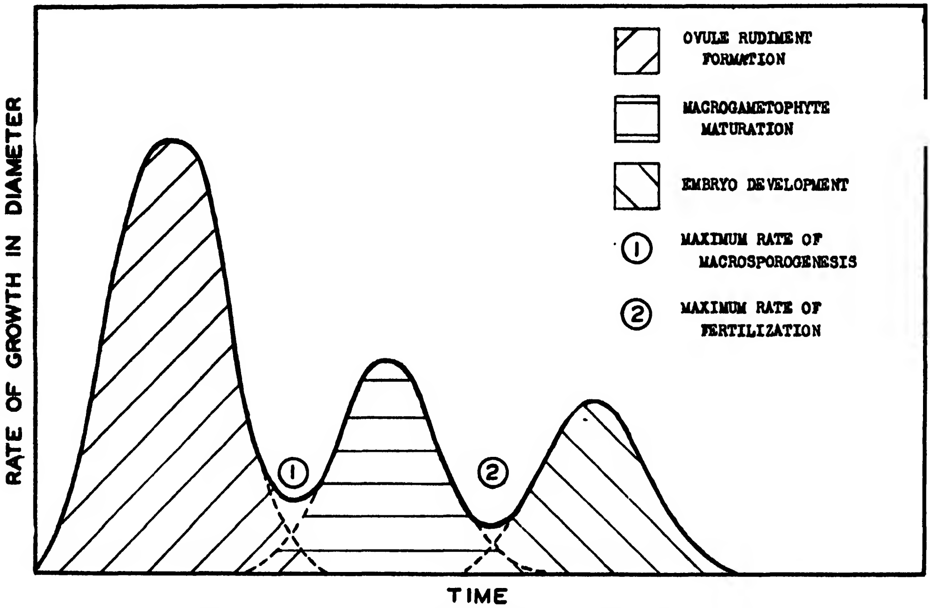


FIG. 9. Generalized curve based on weekly increments of growth in diameter of fruits of *Phalaenopsis*.

ment) with the exception that the first is incorrect in time of initiation since the pollen effect is not segregated from it.

The results, now being prepared for publication, of similar investigations on numerous tropical orchids show highly comparable sequences of events It is not thought, however, that all, or perhaps any, temperate orchids will do likewise. In such forms as *Orchis* (Hildebrand 1863) the ovary is farther advanced at time of pollination in that ovule rudiments are present. The recognition of similar growth cycles in the four genera of the *Cypripedilineae* may be more difficult in that ovule rudiments are present and in that the fairly large ovary cavity, present at time of pollination, allows space for considerable internal development without external manifestations.

Examination of data gathered by other workers on fruit development in forms not members of the *Orchidaceae* almost universally shows a break in the growth curve. Such breaks have been interpreted in three ways. First, they have been ignored and an exponential growth curve has been erected; second, they have been interpreted as an indication that at such a time individual parts of the ovary were growing at slightly different rates; third, they have been considered to indicate a fundamental physiological change. The present study goes beyond the last view in suggesting that there is a close correlation in the orchids between the growth rate as measured externally and the internal state of development.

Lilleland (1931) found a retarded period separating two periods of rapid growth in diameter of the pericarp of the apricot. This is considered characteristic of drupaceous fruits. Since pomes lack this retarded period, its cause has been ascribed to competition for food substances by the fleshy and stony layers of the pericarp. Tukey's (1933) graphs of the development of the cherry fruit show a deceleration of the rate of pericarp growth at the time endosperm and embryo are initiating their grand phase of development. However, it is highly improbable, because of the relative immaturity of the *Phalaenopsis* ovary at the time of pollination, that any close comparison can be drawn between fruit development in other Angiosperms in which it is well known and *Phalaenopsis*. Even in such genera as *Alnus* (Wolpert 1910), in which a period of three or four weeks elapses between pollination and fertilization, ovules, although not ready for fertilization, are present in some stage of development at the time of pollination. Furthermore the embyo sac reaches maturity even though the flower remains unpollinated. In general *Phalaenopsis* and other Angiosperms have pollination and fertilization effects in common, but the growth phases in *Phalaenopsis* lying between pollination and fertilization and having to do with placental proliferation, ovule initiation, and ovule maturation would probably come prior to the growth phase initiated by pollination in the ordinary Angiosperm. To state the assumption in another way: a pollination effect followed immediately by and perhaps indistinguishably united with a fertilization effect would be found in those Angiosperms which possess mature or nearly mature ovules at the time of pollination.

Strictly comparable studies on the growth phases of the ovaries of other Angiosperms are lacking. Likewise it remains to be seen how universal is this type of ovary development in the orchids. The appearance of mature ovules in *Phalaenopsis* long after pollination offers novel opportunities for experimental manipulations (including injection with various substances) at various critical points of its development as can be determined from the configuration of the growth curve.

SUMMARY

The ovary of *Phalaenopsis* has intermittent development.

The presence of the pollinia on the stigma brings about: (1) wilting of the perinath; (2) an initiation of column growth so that the pollinia are enclosed by the edges of the stigmatic cavity; (3) a change in the curvature of the ovary; (4) the initiation of nuclear and cell division in the cells in the inner layer of the ovary wall and in the cells of the placentae; and (5) the initiation of growth in both length and diameter.

Fruit set is determined by the entrance of the pollen tubes into the ovary cavity. At the same time added impetus is given to growth in length and diameter.

Ovary development is marked by three well defined phases of growth in diameter and two in length. The phases of growth in diameter coincide with morphological changes within the ovary, namely: (1) placental proliferation and ovule initiation, (2) preparation of the ovule for fertilization, and (3) embryo growth. The first of the phases of length growth closes at the time of fertilization; the second takes place at the time seeds are maturing. Critical points such as macrosporogenesis and fertilization take place while growth in diameter is near a minimum.

Incompatibility factors are expressed at the close of pollination effects. A type of sterility resulting in premature ripening of the fruit and following failure in macrosporogenesis or of the pollen tubes to reach the ovules was likewise found.

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VITAMIN DEFICIENCIES OF CERATOSTOMELLA

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It is now well established that the growth of many fungi is limited by their inability to make adequate quantities of one or more vitamins or vitamin-like growth substances. Besides being heterotrophic in relation to carbon these organisms exhibit various degrees of heterotrophy for growth substances. Such fungi do not grow or grow poorly in a solution of pure sugar, minerals, and asparagine, but they develop satisfactorily on the addition to this medium of various substances of natural origin, or of one or more chemically pure vitamins. Some of these fungi suffer from complete or major vitamin deficiencies,[1] that is, they make no growth unless the needed vitamins are supplied; others suffer from minor or partial deficiencies, that is, they grow slowly in the absence of the vitamin, but grow much more rapidly if some of the vitamin is present in the medium. Some fungi have a single (partial or complete) deficiency, but others may be afflicted with two or more.

The genus *Ceratostomella* is particularly interesting because it illustrates all of the generalities expressed above when the relation of some of its species to thiamine (vitamin B_1), pyridoxine (vitamin B_6), and biotin (vitamin H) is studied.

The genus *Ceratostomella* includes the causal organism of the Dutch elm disease, the cause of a serious disease of the London plane tree, and some fungi responsible for diseases of a variety of forest trees. Some species are known as the blue-stain fungi.

Through the courtesy of Dr. Carl Hartley and Dr. Caroline T. Rumbold we received cultures of the following species or strains of *Ceratostomella*.

C. ips Rumbold No. 255 associated with *Ips avulsus* in *Pinus echinata* and collected near Asheville, North Carolina (6, 7).

C. ips Rumbold No. 438 A associated with *Ips integer* in *Pinus ponderosa* and collected near Sisters, Oregon (6, 7).

C. pini Munch No. 512 associated with *Dendroctonus fontalis* in *Pinus echinata* and collected near Heleyville, Alabama (6, 7).

* The material contained in this paper was presented at the meetings of the National Academy of Sciences on October 13, 1941, at Madison, Wisconsin.

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[1] Two classes of vitamin deficiencies should be distinguished. One is physiological, an inability of the organism to synthesize a particular vitamin from simple foods and nutrients, for example, from sugar, minerals, and a source of nitrogen; the other is a vitamin deficiency in the food. Only those organisms which are physiologically deficient can suffer from a vitamin deficiency in the food. Schopfer has used the somewhat awkward term auxoheterotrophic to describe organisms physiologically deficient for one or more vitamins, and auxoautotrophic for those which are not deficient. It is the physiological deficiencies which chiefly concern us in this paper.

C. pini Munch No. 416 associated with *Dendroctonus breviconus* in *Pinus ponderosa* and collected near Sisters, Oregon (6, 7).

C. piceaperda Rumbold No. 240 associated with *Dendroctonus piceaperda* in *Picea glauca* and collected in Nova Scotia (7).

C. pseudotsugae Rumbold No. 436 associated with *Dendroctonus pseudotsugae* in Douglas fir and collected near Metaline Falls, Washington (7).

C. montium Rumbold No. 424 associated with *Dendroctonus monticolae* in *Pinus monticola* and collected near Metaline Falls, Washington (8).

Included in the study reported here were cultures of *C. ulmi* and of a *Ceratostomella* isolated from the London plane tree obtained through the kindness of Dr. James M. Walter, and a culture of *C. fimbriata* isolated from sweet potato and supplied by Dr. L. L. Harter.

METHODS AND MATERIALS

The organisms were grown in pure culture in 125 ml. Erlenmeyer flasks containing 25 ml. quantities of a basal solution, solution 1, composed per liter of 50 g. dextrose, 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4 \cdot 7H_2O$, and 2 g. asparagine. To this solution the following trace elements in p.p.m. were added: 0.005 B, 0.02 Cu, 0.1 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo, and 0.09 Zn. The dextrose was Corn Products C.P. The asparagine was purified by treatment with Norit A and recrystallization from redistilled alcohol. All other chemicals were of chemically pure grade.

Merck's synthetic thiamine and pyridoxine were used; pure biotin methyl ester was obtained through the courtesy of Dr. Vincent du Vigneaud and a preparation from the SMA corporation was also used. Other vitamins or special chemicals employed are mentioned later in the text.

The fungi were grown also on agar slants in test tubes, each containing approximately 8 ml. of medium. The basal medium in these cultures consisted of solution 1 plus the mineral supplements solidified with 1.5 per cent Difco agar or 1.5 per cent agar which had been purified by extraction with aqueous pyridine and alcohol as previously described (5).

Pyrex glass thoroughly cleaned with a chromic–sulfuric acid mixture and washed with tap and distilled water was used throughout the experiments. The media were sterilized in an autoclave for 20 minutes at 15 lbs. pressure.

EXPERIMENTS

Response to Peptone. The Ceratostomellas grow readily on media supplemented with extracts of various natural products, for example, on malt agar, on agar containing an infusion of tree bark or on a thiamine-peptone agar (4). Preliminary investigation demonstrated that most of the 10 species or strains grew very poorly, or not at all, in a solution of dextrose, minerals,

asparagine, and thiamine, but grew quite well in the same medium containing peptone. This is illustrated in table 1. In the experiment summarized

TABLE 1. *Average dry weight in mg. of duplicate cultures of the Ceratostomellas indicated in a mineral-dextrose solution containing asparagine and thiamine, and in the same solution plus peptone*
Temperature 26° C., time of growth in each passage 15 days.

Additions to 25 ml. of solution 1	*C. piceaperda* No. 240	*C. ips* No. 255	*C. pini* No. 416	*C. montium* No. 424	*C. pseudotsugae* No. 436	*C. ips* No. 438	*C. pini* No. 512	*C. ulmi*
10 μ g. thiamine first passage	1.8	0.2	11.3	0.2	57.9	2.4	1.6	0.1
10 μ g. thiamine and 25 mg. peptone 1st passage	44.5	27.3	52.7	14.4	76.4	39.4	38.2	16.9
10 μ g. thiamine 2nd passage	0.3	0.0	0.9	0.0	52.6	1.8	1.9	0.0
10 μ g. thiamine and 25 mg. peptone 2nd passage	45.6	25.4	52.5	13.3	66.9	39.4	38.4	18.5

there, duplicate cultures, each containing 25 ml. of solution 1 plus 10 μg. of thiamine or plus 10 μg. of thiamine and 25 mg. of neo-peptone, were inoculated from stock cultures grown on a thiamine-peptone agar. After 8 days growth at 26° C. sub-cultures were made into corresponding solutions for a second passage. Dry weights for both passages were determined after 15 days growth. *C. pseudotsugae* made considerable growth in the basal solution containing thiamine, but its growth was decidedly improved by the addition of peptone. The growth of the other 7 strains or species in the basal solution plus thiamine was limited to a milligram or two and was increased many times by the addition of peptone. It appeared, therefore, that peptone supplied these species of *Ceratostomella* with something necessary for growth which was not present in solution 1 plus thiamine.

Response to Thiamine, Pyridoxine, and Biotin. Further investigation in which 11 vitamins[2] and a number of amino acids were used, in various combinations, indicated that deficiencies of thiamine, pyridoxine, and biotin were primarily concerned in determining the growth of the Ceratostomellas studied.

The response of these fungi to the three vitamins was determined by growing each organism in 25 ml. of the basal solution and in the same medium plus biotin, thiamine, pyridoxine, biotin and thiamine, biotin and pyridoxine, pyridoxine and thiamine, or all three vitamins. The quantity of

[2] See footnote 3.

these vitamins used per flask was 0.1 μg. of biotin, 10 mμ moles of thiamine, and 50 mμ moles of pyridoxine. Each organism was grown also in the basal solution plus 0.3 g. of malt extract per flask. The experiment was performed in triplicate. The liquid cultures were inoculated from stock cultures on thiamine-peptone agar and incubated at 20° C. in the dark. Dry weights were determined after various periods of time, depending upon the rapidity of growth, as follows:

C. pseudotsugae No. 436, *C. ips* No. 438, after 8 days; *C. piceaperda* No. 240, *C. ips* No. 255, *C. pini* No. 416, *C. montium* No. 424, after 12 days; *C. pini* No. 512, after 14 days; *C. ulmi, C. fimbriata*, and the *Ceratostomella* from the London plane, after 20 days (table 2).

TABLE 2. *Average dry weight in mg. of triplicate cultures of the Ceratostomellas indicated in a basal mineral-dextrose solution containing asparagine to which thiamine, pyridoxine, and biotin were added alone or in combination*

Temperature of incubation 20° C. Time of growth ranged from 8 to 20 days; see text for details. First passage. Compare with table 3.

Additions to 25 ml. of solution 1	None	0.1 μg. biotin	50 mμ moles pyridoxine	10 mμ moles thiamine	pyridoxine and biotin	pyridoxine and thiamine	biotin and thiamine	pyridoxine, biotin, and thiamine	0.3 g. malt extract
C. pseudotsugae, No. 436	6.2	6.5	5.2	51.4	6.7	66.6	51.6	46.5	92.2
C. ips, No. 438	4.3	5.4	0.1	1.0	25.2	0.3	18.4	39.9	92.4
C. piceaperda, No. 240 ...	0.2	2.8	3.3	0.2	8.2	17.6	16.9	27.5	48.4
C. ips, No. 255 ...	no growth	no growth	no growth	no growth	1.9	0.1	0.1	52.6	51.5
C. pini, No. 416 .	0.2	2.2	0.1	0.1	1.2	0.1	35.6	34.9	32.0
C. montinum, No. 424	0.1	0.8	1.4	0.1	10.7	3.2	3.3	33.2	50.1
C. pini, No. 512 ...	1.3	1.2	0.1	0.3	0.9	0.2	37.7	41.7	57.4
C. ulmi	no growth	no growth	25.1	no growth	24.0	25.2	no growth	25.2	119.7
C. fimbriata ...	2.1	4.5	0.1	32.4	0.6	60.6	59.9	103.6	91.6
C. from London plane ...	0.1	no growth	no growth	1.5	1.4	32.0	18.5	26.0	54.2

Sub-cultures from the growth in the first passage in liquid cultures were made into similar solutions for a second passage. This was in order to de-

crease the effect of whatever vitamins might have been present in the original inoculum which came from cultures grown on thiamine-peptone agar.

Each organism in the second passage (table 3) was grown also in the basal solution plus 11 vitamins and 21 amino acids[3] and in the basal solution plus 50 mμ moles of pyridoxine, 10 mμ moles of thiamine, and 19 mg. of neo-peptone per flask. For inoculating these cultures transfers from growth in the first passage in the basal solution was used for No. 436, No. 438, No. 424, No. 512, and *C. fimbriata,* from the basal solution supplemented with biotin for No. 240, No. 416, and *C. ulmi,* and from the basal solution supplemented with pyridoxine and thiamine for No. 255 and the *Ceratostomella* from the London plane.

Dry weights for the second passage (table 3) were determined after the same time intervals as in the first passage except for *C. ulmi, C. fimbriata,* and the *Ceratostomella* from the London plane, which were grown for 18 days only.

In addition the 10 organisms were grown in triplicate in test tubes on agar slants containing the basal solution solidified with 1.5 per cent purified agar and in the same medium plus per tube the same quantities and combinations of thiamine, pyridoxine, and biotin used in the liquid cultures. The cultures were incubated in the dark at 20° C. After 11 days sub-cultures were made to corresponding media. The results in the second passage on agar did not differ significantly from those observed in the first passage.

The results on the agar cultures and in the liquid media were as follows:

C. ips, No. 438, did not grow on the basal medium solidified with 1.5 per cent purified agar. The addition of thiamine or pyridoxine alone or together was of no benefit. The addition of biotin, biotin and thiamine, biotin and pyridoxine or of biotin, pyridoxine, and thiamine permitted good growth (fig. 1). It appeared from these results that *C. ips* No. 438 suffered from a complete or nearly complete biotin deficiency.

This conclusion was confirmed by experiments in liquid culture, which showed in addition that partial deficiencies for pyridoxine and thiamine probably exist for this organism. Very little growth occurred in the basal solution (tables 2, 3). The addition of biotin increased the growth several times. The quantity of pyridoxine and of thiamine used appeared to be somewhat injurious, for less dry matter was obtained in the cultures supple-

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[3] The following amino acids were added in μg. per flask: d arginine 10, dl alanine 3, l aspartic acid 10, d glutamine 22, glycine 3, β alanine 80, l leucine 16, dl lycine HCL 32, dl norleucine 16, serine 16, dl theonine 16, dl valine 16, d cysteine HCl 16, l histidine HCl 32, l hydroxyproline 16, dl methionine 16, dl phenylalanine 16, l proline 16, l tryptophane 32, tyrosine 8, thyroxine 8.

The following vitamins were added per flask: ascorbic acid 12 μg., pimelic acid 6 μg., para-amino-benzoic acid 6 μg., vitamin K_1 6 μg., calcium pantothenate 6 μg., lactoflavin 10 mμ moles, nicotinamide 10 mμ moles, m-inositol 1 mg., pyridoxine 50 mμ moles, thiamine 20 mμ moles, biotin 0.1 μg.

mented with thiamine or pyridoxine than in the basal solution. Pyridoxine and biotin together or thiamine and biotin together were 3 to 5 times as effective as biotin alone while the best growth was obtained with all three vitamins present in the medium. Growth in the media containing biotin was distinctly better in the second passage than in the first.

C. ulmi grew on the agar medium satisfactorily only in those tubes which contained pyridoxine. On the basal medium or on the basal medium containing thiamine or biotin, or a combination of the two, the development was slight (fig. 1). *C. ulmi* apparently has a complete pyridoxine deficiency.

These observations were supported by the results in liquid culture

TABLE 3. *Average dry weight in mg. of triplicate cultures of the Ceratostomellas indicated in a basal mineral-dextrose solution containing asparagine to which thiamine, pyridoxine, and biotin were added alone or in combination*

Temperature of incubation 20° C. Time of growth ranged from 8 to 18 days; see text for details. Second passage. Compare with table 2. See footnote No. 3 for the 11 vitamins and 21 amino acids used.

Additions to 25 ml. of solution 1	None	0.1 μg. biotin	50 mμ moles pyridoxine	10 mμ moles thiamine	pyridoxine and biotin	pyridoxine and thiamine	biotin and thiamine	biotin, pyridoxine and thiamine	Eleven vitamins and 21 amino acids	pyridoxine, thiamine, 19 mg. neopeptone
C. pseudotsugae, No. 436	5.1	4.5	10.3	69.9	12.6	64.5	78.5	73.7	82.4	85.4
C. ips, No. 438	2.7	16.9	1.0	0.8	65.0	2.2	68.6	77.8	76.2	31.9
C. piceaperda, No. 240	0.7	4.4	13.5	0.5	16.0	26.9	18.3	43.8	59.8	61.0
C. ips, No. 255	no growth	no growth	no growth	no growth	1.3	no growth	no growth	55.8	49.2	44.4
C. pini, No. 416	0.3	1.2	no growth	no growth	1.0	no growth	57.7	60.9	57.6	46.4
C. montinum, No. 424	0.3	3.5	0.6	0.5	3.4	2.3	0.2	48.1	55.1	44.1
C. pini, No. 512	0.1	0.6	0.4	0.4	0.8	0.1	34.2	39.2	64.8	40.8
C. ulmi	no growth	no growth	36.8	no growth	30.7	28.8	no growth	29.4	42.3	56.9
C. fimbriata	0.7	0.7	0.5	95.9	0.7	131.4	130.8	127.8	62.7	65.6
C. from London plane	0.3	0.1	0.2	14.6	0.1	34.9	37.9	40.2	44.2	68.2

(tables 2, 3). *C. ulmi* did not grow in the basal medium and the addition of thiamine or of biotin, alone or together, had no effect. The growth with

FIG. 1. Growth of various species of *Ceratostomella* on a basal mineral—dextrose medium containing asparagine and 1.5 per cent purified agar supplemented as follows: (1) no addition; (2) biotin; (3) pyridoxine; (4) thiamine; (5) biotin and pyridoxine; (6) pyridoxine and thiamine; (7) thiamine and biotin; (8) pyridoxine, thiamine, and biotin. A, *C. ips* No. 438; B, *C. ulmi; C, C. fimbriata.* Age of cultures 26 days.

pyridoxine, pyridoxine and thiamine, pyridoxine and biotin, and with all three vitamins was approximately the same, indicating under these conditions no partial deficiencies for biotin or thiamine.

Attempts to substitute nicotinamide, calcium pantothenate, m-inositol, or para-amino benzoic acid for pyridoxine were unsuccessful.

C. fimbriata grew very little in the basal agar medium and the addition of pyridoxine or of biotin alone and together had little effect. When thiamine was added growth was quite satisfactory (fig. 1); more rapid development occurred in those media supplemented with thiamine and pyridoxine, thiamine and biotin, or with the three vitamins. *C. fimbriata* appeared to have a complete deficiency for thiamine, and partial deficiencies for pyridoxine and biotin.

These conclusions were confirmed by the liquid cultures (tables 2, 3). Little growth was obtained in the basal medium or in the basal medium plus biotin or pyridoxine singly or together. Good growth was obtained on the addition of thiamine and was increased when thiamine and pyridoxine or thiamine and biotin were used as supplements. The best growth was obtained when all three vitamins were present.

The *Ceratostomella* from the London plane tree resembled *C. fimbriata* in its vitamin deficiencies. It grew less rapidly and may suffer from more marked pyridoxine and biotin deficiencies than *C. fimbriata* (tables 2, 3).

C. piceaperda, No. 240, grew slowly on the basal agar medium. Little growth was visible after 5 days. After 7 days colonies 2–5 mm. in diameter had developed and after 10 days the colonies averaged 12 mm. in diameter. The addition of thiamine to the basal medium had some, though little, beneficial effect on the early growth, but the addition of biotin or pyridoxine materially increased the rapidity of growth (fig. 2). The tubes containing biotin or pyridoxine after 5 days had colonies from 33 to 50 mm. in diameter and after 10 days the agar slopes were entirely covered with mycelium. The most rapid growth occurred in the tubes containing all three vitamins. The cultures on agar indicated that *C. piceaperda* suffers from partial deficiencies for biotin and pyridoxine. When these deficiencies were satisfied the addition of thiamine had a further beneficial effect.

C. piceaperda grew very little in the basal solution and the addition of thiamine had little effect (tables 2, 3). Biotin increased growth somewhat but pyridoxine had a much greater effect. Pyridoxine and biotin together were about as effective as pyridoxine alone and about equal to the mixture of biotin and thiamine. Pyridoxine and thiamine together were still more effective and the three vitamins combined were most effective. These comments are based on the results of the second passage (table 3).

C. piceaperda grew on the basal agar medium and acted as though it suffered chiefly from partial biotin and pyridoxine deficiencies. In liquid culture it grew little or not at all in the basal medium and appeared to have a complete deficiency for biotin and pyridoxine and a partial thiamine deficiency. The apparent differences between the growth of *C. piceaperda*


A
B
1 2 3 4 5 6 7 8
C
1 2 3 4 5 6 7 8
1 2 3 4 5 6 7 8

on agar and in liquid culture may be the result of more rapid development on agar. It was noted that the relative growth in the second passage in the liquid cultures at the end of 12 days was much the same as that observed on agar at the end of 6 days.

C. pseudotsugae, No. 436, grew slowly on the basal agar medium. At the end of 5 days this fungus had developed colonies 19 mm. in diameter and after 12 days the colonies were 39 mm. in diameter. The addition of biotin did not increase the rapidity of growth but pyridoxine had some beneficial effect and thiamine was distinctly beneficial. The combination of biotin and pyridoxine was more beneficial than pyridoxine alone. Good growth was obtained also on the media containing pyridoxine and thiamine, biotin and thiamine, or all three vitamins (fig. 2). *C. pseudotsugae* seemed to have partial deficiencies for thiamine and pyridoxine.

In the first passage in liquid culture there was no evidence for a pyridoxine deficiency, but in the second passage partial deficiencies for thiamine and pyridoxine were indicated by the increased growth obtained when these vitamins were supplied (table 3).

C. pini, No. 512, did not grow on the basal agar medium and the addition of biotin, of pyridoxine, of thiamine, of biotin and pyridoxine together, or of pyridoxine and thiamine together had little or no effect (fig. 2). The addition of biotin and thiamine or of all three vitamins permitted good growth. From these results it appeared that this fungus suffers from complete biotin and thiamine deficiencies.

The liquid cultures confirmed this assumption. Little growth was obtained in any of the solutions except those supplemented with both biotin and thiamine or all three vitamins. The somewhat greater growth obtained when all three vitamins were present suggests that there may be also a partial pyridoxine deficiency (table 3).

C. pini, No. 416, responded much as did the isolation of the same species from *Pinus echinata*. It grew somewhat more rapidly and differed somewhat in gross morphology (tables 2, 3).

C. ips, No. 255, did not grow on the basal agar medium. The addition of biotin produced a small amount of growth but thiamine or pyridoxine was entirely ineffective. When the vitamins were added to the basal medium in pairs thiamine and pyridoxine were entirely ineffective and the growth with biotin and thiamine or with biotin and pyridoxine was about the same as

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Explanation of figure 2

FIG. 2. Growth of various species of *Ceratostomella* on a basal mineral—dextrose medium containing asparagine and 1.5 per cent purified agar supplemented as follows: (1) no addition; (2) biotin; (3) pyridoxine; (4) thiamine; (5) biotin and pyridoxine; (6) pyridoxine and thiamine; (7) thiamine and biotin; (8) pyridoxine, thiamine, and biotin. A, *C. piceaperda*, No. 240; B, *C. pseudotsugae*, No. 436; C, *C. pini* No. 512; D, *C. ips* No. 255. Age of *C. pseudotsugae* 12 days; others, 26 days.

with biotin alone. When all three vitamins were added the growth was quite satisfactory. *C. ips,* No. 255, appeared to have complete or nearly complete deficiencies for biotin, thiamine, and pyridoxine, and in order to obtain good growth all three vitamins had to be added to the medium (fig. 2).

These results were essentially duplicated in the liquid cultures (tables 2, 3). In the second passage 55.8 mg. of mycelium were produced in the presence of all three vitamins while the maximum obtained with any single one or combination of two was 1.3 mg.

It is interesting to contrast this strain of *C. ips* with No. 438 which showed a single major deficiency—one for biotin.

C. montium, No. 424, closely resembled *C. ips*, No. 255, in its vitamin needs on the agar media. It appeared to have complete or nearly complete deficiencies for all three vitamins. This was true also for the liquid cultures (tables 2, 3). Little growth occurred in the basal solution, biotin improved the situation a little, but the growth was insignificant until all three vitamins were added.

The results given above indicate that these 10 fungi, all belonging to the genus *Ceratostomella,* have 7 distinct combinations of vitamin deficiencies under the conditions of our experiments. These may be tabulated as follows:

TABLE 4. *Vitamin deficiencies of 10 species or strains of Ceratostomella*

Note that the 10 fungi show 7 different types of deficiencies for the 3 vitamins. For *C. piceaperda* the partial deficiencies for biotin and pyridoxine are the more marked.

Fungus	Deficiency for		
	Biotin	Pyridoxine	Thiamine
C. ips No. 438	complete	partial	partial
C. ulmi	none	complete	none
C. fimbriata	partial	partial	complete
C. from London plane	partial	partial	complete
C. piceaperda No. 240	partial	partial	partial
C. pseudotsugae No. 436	none	partial	partial
C. pini No. 512	complete	partial	complete
C. pini No. 416	complete	partial?	complete
C. ips No. 255	complete	complete	complete
C. montium No. 424	complete	nearly complete	nearly complete

Synthesis of Vitamins by Ceratostomella. All the available evidence indicates that each living organism requires many vitamins. An organism which shows a deficiency for one is capable of synthesizing the others from the more elementary foods and nutrients, for example, from sugar, minerals, and a nitrogen source. It seemed worth while to test this assumption for the organisms and the vitamins concerned in this study.

C. fimbriata grew in a medium supplemented with thiamine. Is it capable of making biotin and pyridoxine from sugar, minerals, and asparagine (and thiamine)? The vitamin deficiency for *C. ulmi* is pyridoxine. Can it synthesize thiamine and biotin?

The procedure in answering these questions for *C. fimbriata* was as follows:

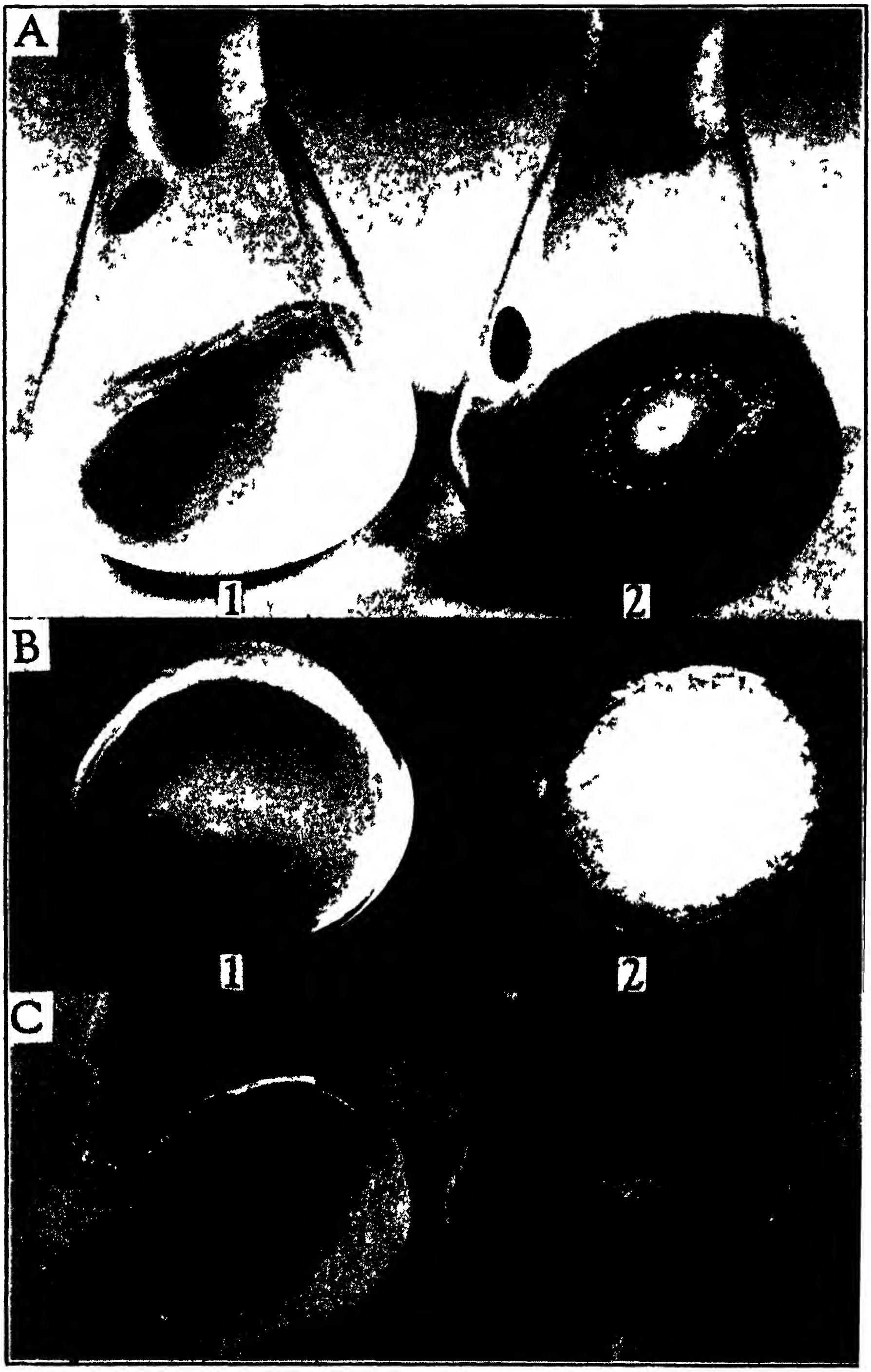
C. fimbriata was grown in 25 ml. quantities of the basal solution plus 10 mμ moles of thiamine per flask. At the end of 9 days and without the removal of the mycelium sufficient purified agar was added to each flask to make a 1.5 per cent solution and the solution was sterilized. The agar medium in some flasks was inoculated with *C. ulmi,* in others with *C. ips* No. 438, and in others with *C. montium* No. 424. Since the original medium contained thiamine but no pyridoxine, growth of *C. ulmi* would show that *C. fimbriata* had synthesized pyridoxine. Similarly growth of *C. ips* No. 438 would show that *C. fimbriata* had made biotin. *C. montium* No. 424 has complete deficiencies for all three vitamins and its growth on a medium to which thiamine only had been originally added would demonstrate that *C. fimbriata* had synthesized both pyridoxine and biotin. The results showed clearly the synthesis of pyridoxine and biotin by *C. fimbriata* from sugar, minerals, and asparagine (and thiamine) (fig. 3). By an analogous procedure the synthesis of vitamins by other species of *Ceratostomella* was determined as shown in the following table. All tests were positive.

TABLE 5. *Various species or strains of Ceratostomella tested for synthesis of vitamins*

The vitamins tested for and the organisms used in the bio-assay are given. The results of all tests were positive.

Fungus and culture solution tested	Tested for	Tested with
C. fimbriata grown in soln. 1 plus thiamine	pyridoxine biotin pyridoxine and biotin	*C. ulmi* *C. ips* No. 438 *C. montium* No. 424
Ceratostomella from London plane grown in soln. 1 plus thiamine	pyridoxine biotin pyridoxine and biotin	*C. ulmi* *C. ips* No. 438 *C. montium* No. 424
C. ulmi grown in soln. 1 plus pyridoxine	biotin thiamine biotin and thiamine	*C. ips* No. 438 *C. fimbriata* *C. montium* No. 424
C. ips No. 438 grown in soln. 1 plus biotin	pyridoxine thiamine	*C. ulmi* *C. fimbriata*
C. pini No. 512 grown in soln. 1 plus biotin and thiamine	pyridoxine pyridoxine	*C. ulmi* *C. montium* No. 424
C. pini No. 416 grown in soln. 1 plus biotin and thiamine	pyridoxine pyridoxine	*C. ulmi* *C. montium* No. 424

Other Vitamin Deficiencies. It is possible to grow any of the 10 fungi studied in a basal medium supplemented with thiamine, pyridoxine and biotin. Do these three vitamins satisfy all the vitamin deficiencies of all these organisms? It is probable that they do not. It is probable that some of these organisms suffer from partial deficiencies for other unidentified vitamins.


A
1
2
B
1
2
C

This is suggested by the development of perithecia on the basal agar medium supplemented with the three vitamins as compared with that described by Rumbold on malt agar. Several of the fungi did not form perithecia on our synthetic media and none developed these bodies so rapidly as has been described for their formation media containing natural supplements. Of course, we cannot on the basis of our present knowledge ascribe the slowness or the failure of perithecial development to deficiencies for vitamins other than thiamine, biotin, and pyridoxine. A number of other factors may be concerned. However, the perithecial development indicates that the basal medium supplemented with the three vitamins is probably not optimal for all these organisms and partial deficiencies for one or more additional vitamins may be concerned.

Further evidence for partial deficiencies of additional vitamins is found in the relative growth of some of these fungi in the basal medium supplemented with the three vitamins and in media containing additional vitamins or natural products.

For example, *C. ulmi* produced a dry weight of 25.2 mg. in the basal medium plus thiamine, pyridoxine, and biotin, but with malt extract the yield was 119.7 mg. (table 2). In the basal medium containing thiamine, pyridoxine, and peptone, the yield was 56.9 mg., as compared to 29.4 mg. in the basal medium containing thiamine, biotin, and pyridoxine (table 3). One explanation for the benefit noted from malt extract and from peptone on the growth of *C. ulmi*, which should be investigated, is that these natural products supply additional vitamins.

The growth of *C. piceaperda* No. 240 was improved by the addition of calcium pantothenate, nicotinamide, and para-amino benzoic acid to a basal solution containing biotin, thiamine, pyridoxine, and m-inositol. Still greater improvement was observed with the further addition of lactoflavin, ascorbic acid, pimelic acid, and glutamine. This suggests that partial deficiencies for other vitamins exist for this organism. In fact, greater growth was obtained in a medium supplemented with additional vitamins or with some natural product for all the *Ceratostomellas* except *C. ips* No. 255, *C. pini* No. 416, and *C. fimbriata*.

The addition to the basal medium of 11 vitamins and 21 amino acids did not improve the growth of No. 438, No. 255, No. 416, *C. fimbriata*, and the *Ceratostomella* from the London plane (table 3), as compared to that in the basal medium plus biotin, pyridoxine, and thiamine. However, the basal

Explanation of figure 3

FIG. 3. Production of biotin and pyridoxine by *C. fimbriata*. (1) basal medium containing thiamine solidified with purified agar; (2) same medium in which *C. fimbriata* was grown also solidified with purified agar. A, inoculated with *C. ips* No. 438; B, inoculated with *C. ulmi*; C, inoculated with *C. montium* No. 424.

medium plus malt extract was more effective than the basal medium plus thiamine, biotin, and pyridoxine for No. 438 and the *Ceratostomella* from the London plane. Furthermore, the beneficial effect of the malt extract of *C. ulmi* was much greater than that of the 11 vitamins and 21 amino acids. This suggests that *C. ips* No. 438, the *Ceratostomella* from the London plane, and *C. ulmi* suffer from deficiencies not supplied by the 11 vitamins and 21 amino acids.

The possibility that partial deficiencies for other vitamins than biotin, thiamine, and pyridoxine exist for some of these organisms and the conditions for good perithecial development should be investigated further.

Effect of Vitamins on Gross Morphology. The nature of the vitamins supplied influenced the pigment production and the character of the growth in several instances. For example, *C. fimbriata* developed on agar a white cottony mycelium when supplied with thiamine only, but with thiamine and biotin, with thiamine and pyridoxine, or with all three vitamins the mycelium was moist with a white frosting. In liquid culture after 8 days the same organism in the solution supplemented with thiamine showed a single submersed mycelial colony about 1 cm. in diameter, but with thiamine and pyridoxine, thiamine and biotin, or all three vitamins the surface of the liquid was covered with a dry torula-like growth in addition to the submersed mycelium.

C. ips No. 438 formed a dry mycelium on agar supplemented with biotin, while on agar supplemented with biotin and thiamine, biotin and pyridoxine, or with all three vitamins the growth was moist. The casual observer might easily have concluded that two different organisms were concerned.

Time did not permit a detailed investigation of these differences, some of them doubtless associated with the relative abundance of conidia. It is worth recording, however, that the supply of vitamins did markedly influence the gross morphology of some of these fungi.

Presence of Biotin, Pyridoxine and Thiamine in Cotton Batting and in Agar. In the course of an experiment not reported in detail here it was noted that *C. ips* No. 255 grew vigorously in one flask of three, which contained the basal solution plus thiamine and calcium pantothenate. Since this fungus suffers from complete thiamine, biotin, and pyridoxine deficiencies, it was evident that the last two vitamins must have been fortuitously introduced into this flask. Investigation showed that water had dripped during sterilization from the roof of the autoclave on the cotton stopper of this flask and percolated into the culture medium. Cotton batting was therefore investigated for biotin, pyridoxine, and thiamine.

A quantity of cotton batting was moistened with distilled water and autoclaved. The liquid was pressed out and made up so that 1 ml. of the

extract was equivalent to 1 g. of the cotton batting. No attempt was made to make a thorough and complete extraction of the cotton. The extract contained 12.2 mg. dry matter per ml.

To determine the presence of vitamins in this extract, tubes of the basal medium plus 1.5 per cent purified agar were prepared. To some tubes 1 ml. of the cotton extract was added. Tubes of the basal medium and of the basal

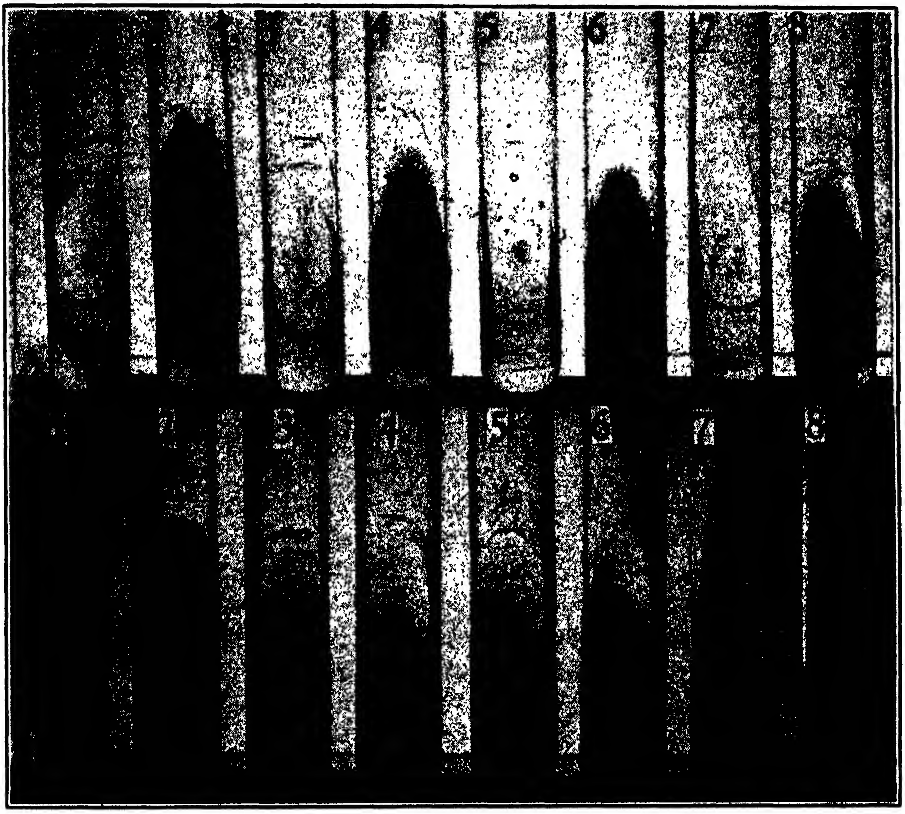


FIG. 4. Presence of biotin, pyridoxine, and thiamine in cotton extract and unpurified agar. A(1) basal medium with purified agar inoculated with *C. ips* No. 4338; A(2) same with extract from 1 g. cotton batting; A(3) basal medium inoculated with *C. ulmi;* A(4) same with cotton extract; A(5) basal medium inoculated with *C. fimbriata;* A(6) same with cotton extract; A(7) basal medium inoculated with *C. ips* No. 255; A(8) same with cotton extract. B(1–8) purified agar compared with unpurified agar.

medium plus the cotton extract were inoculated in triplicate with *C. ips* No. 438 to test for biotin, with *C. ulmi* to test for pyridoxine, with *C. fimbriata* to test for thiamine, and with *C. ips* No. 255 which grows well only when all three vitamins are present. The results (fig. 4) demonstrated that the cotton extract contained per ml. sufficient biotin, pyridoxine, and thiamine to give maximum or nearly maximum effects with these four species of *Ceratosmella.*

The cotton extract was used also in solution cultures. Growth of *C. ips* No. 438, of *C. ulmi*, of *C. montium* No. 424, and of *C. ips* No. 255 in the basal medium plus the cotton extract demonstrated the presence of thiamine, pyridoxine, and biotin in this material (table 6). The growth of *C. ips* No. 438 in the basal solution plus the extract of 3 g. of cotton was superior to that obtained in any other medium, including malt extract.

TABLE 6. *The effect of an extract of cotton batting on the growth of the Ceratostomellas indicated*

Grown 14 days at 20° C. The average dry weight of triplicate cultures is given in mg.

Additions to 25 ml. of soln. 1	*C. ips* No. 438	*C. ulmi*	*C. montium* No. 424	*C. ips* No. 255	*C. piceaperda* No. 240
10 mμ moles thiamine	0.0	0.0	0.0	0.0	0.1
10 mμ moles thiamine plus extract 0.3 g. cotton	62.0	5.7	3.3	2.8	43.9
10 mμ moles thiamine plus extract 1.5 g. cotton	93.6	9.3	8.3	10.2	74.7
Extract 1.5 g. cotton	121.9	8.3	9.2	7.7	20.6
Extract 3.0 g. cotton	135.1	8.9	13.9	11.7	27.2
10 mμ moles thiamine, 0.01 μg. biotin, 50 mμ moles pyridoxine	10.6	9.2	17.6	11.6	50.5

We cannot be certain that these vitamins were present in the cotton boll and were not introduced in some way after the cotton was picked. The probability is that the former assumption is correct. In any event it is clear that cotton batting, so freely used as a laboratory material, may supply various vitamins, including biotin, pyridoxine, and thiamine.

Difco agar also contains all three vitamins. This is demonstrated in figure 4. The four organisms used in the experiment on cotton extract were grown on the basal medium solidified on the one hand with 1.5 per cent purified agar and on the other with 1.5 per cent unpurified Difco agar. Each tube contained about 0.12 g. of dry agar. Although the greater growth of *C. ulmi* and of *C. fimbriata* on the unpurified agar demonstrated the presence of pyridoxine and thiamine, there was not sufficient of these vitamins in the amount of agar used to give a maximum effect. *C. ips* No. 438 grew quite well on the unpurified agar, showing the presence of sufficient biotin to produce nearly a maximum effect on this fungus.

DISCUSSION

Earlier work on the nutrition of *Ceratostomella* furnishes little information on the nutritional requirements of the genus. Hedgcock (3) cultivated

a considerable number of species of *Ceratostomella* and found a pine decoction agar made from bark and sap wood to be an excellent medium, considerably superior to an agar made from heart wood. Schwarz (9) states that *C. ulmi* did not grow on a nutrient base which contains sugar as the only source of carbon. He grew this organism also on media containing a variety of natural products. It is clear now that *C. ulmi* failed to grow in some of the media used by Schwarz because the medium lacked pyridoxine and not because sugar was the only source of carbon. Clinton and McCormick (2) grew *C. ulmi* on a variety of natural media including elm twigs, cherry agar, plugs of potato and carrot, grains of wheat, oats and barley, sterilized liver, elm bark, and elm wood. Evidently all of these materials contained sufficient pyridoxine to permit growth. Rumbold (6, 7, 8) used chiefly a malt extract agar in the cultivation of the species of *Ceratostomella* she studied. She (7) found *C. pini* grew abnormally on a mineral—dextrose medium containing 1.5 per cent agar and either sodium caseinate or peptone and did not grow on the same medium containing asparagine or urea. It seems probable that lack of sufficient thiamine was partly responsible for these results. Rumbold (7) reported that *C. pseudotsugae* grew well on a mineral—dextrose medium containing 1.5 per cent agar and urea, asparagine, sodium caseinate, or peptone. We found that this organism grew on an agar medium to which no vitamins were added, especially if Difco agar rather than purified agar was used. Taylor-Vinje (10) grew *C. montium* on a corn meal decoction solidified with agar. Bramble and Holst (1) grew *C. pini* on a malt agar or malt broth. Our results show that the advantage of media containing natural products for the cultivation of *Cerastomella* is probably due to the vitamins supplied by the natural supplements.

Our results are of interest also because of the diversity of vitamin deficiencies evidenced by representatives of a single genus. It seems probable that a study of other members of this genus would add to this diversity. We might, for example, find a species which suffers from complete deficiencies for more vitamins than biotin, pyridoxine, and thiamine; one which would not grow even if all three of these vitamins were supplied.

Attention should be called to the vitamin relations of different isolations of the same species. The two isolations of *C. pini*, No. 416 and No. 512, were much alike in their vitamin deficiencies but the two isolations of *C. ips* No. 438 and No. 255, were decidedly different. The former (No. 438) was marked by a complete biotin deficiency and partial deficiencies for pyridoxine and thiamine, while the latter (No. 255) showed complete deficiencies for all three vitamins. Evidently these differences in vitamin deficiencies did not affect the characters differentiating the species.

Our observations are of interest also because of the deficiencies for pyridoxine shown by these organisms. Reports of pyridoxine deficiencies for

filamentous fungi have not hitherto appeared, yet three of the ten *Ceratostomellas* we studied showed a complete pyridoxine deficiency and seven a partial pyridoxine deficiency. *C. ulmi* is of special interest in this connection because it suffers from a pyridoxine (complete) deficiency only. This suggests that it might be used as a bio-assay for this vitamin. However, the character of its growth makes dry weight determinations tedious, and photoelectric determinations of the growth of this organism were not entirely satisfactory. Furthermore, it appears to have unidentified partial deficiencies which markedly affected its growth. What influence this might have on the use of *C. ulmi* for the quantitative determination of pyridoxine in natural products would need further study.

These experiments emphasize also the importance of partial vitamin deficiencies in limiting growth. A complete deficiency causes a marked and dramatic effect. If the vitamin is absent from the medium no growth occurs; its addition, if a single complete deficiency is concerned, permits growth. It is an all or none effect. On the other hand, partial deficiencies permit slow growth to take place in the absence from the medium of the vitamin concerned and its addition merely increases the rate of growth. Partial deficiencies are less obvious in their effects than complete deficiencies. Nevertheless, they both influence the rate and the character of the development of an organism.

Because of the possible influence of partial vitamin deficiencies the occurrence of growth on any given medium may not be taken as a priori evidence for eliminating vitamins as limiting factors. Seven of the ten species or strains studied in the experiments described in this paper were found to suffer from partial deficiencies of thiamine, pyridoxine, or biotin. Furthermore, some evidence for partial deficiencies of unidentified vitamins was found for two of the three remaining organisms. For this genus partial deficiencies are of general occurrence and considerable importance.

The interrelation between the various vitamins presents an interesting situation. *C. fimbriata*, for example, grows very little in the basal medium. The addition of thiamine (table 3) results in a great increase in growth and the synthesis of both biotin and pyridoxine (table 5). However, maximum growth is not obtained in the thiamine medium apparently because the organism does not synthesize enough biotin and pyridoxine, since the addition of biotin or pyridoxine or both to the thiamine medium results in greater growth. The situation is somewhat puzzling. If the biosynthesis of either biotin or pyridoxine limits growth in the thiamine medium then we should expect the addition of one to increase the growth and the other to be ineffective. If the synthesis of both biotin and pyridoxine is the limiting factor in the thiamine medium then it should be necessary to add both before any marked effect is obtained. However, both the combination of thiamine and pyridoxine and of thiamine and biotin are more effective than thiamine

alone. The accumulated evidence from other sources on the specificity of the vitamins would not encourage us to believe that pyridoxine takes the place of biotin or vice versa. We might assume that an increase in the supply of biotin in the presence of thiamine makes the synthesis of more pyridoxine possible and, on the other hand, an increased supply of pyridoxine permits a greater production of biotin. It is possible also that one vitamin is utilized more effectively when others are present in adequate amounts. A similar situation exists in the relation of thiamine and pyridoxine to the growth of *C. ips* No. 438 (table 3) in a medium containing biotin.

SUMMARY

Ten species or strains of *Ceratostomella* were grown in a basal mineral-dextrose medium containing asparagine and in the same medium supplemented with biotin, pyridoxine, or thiamine singly and in combination. All the fungi suffered from complete or partial vitamin deficiencies and 7 different combinations of deficiencies were found. The use of media containing natural products for the cultivation of these fungi is due to their vitamin deficiencies. Some evidence is presented for the existence of deficiencies for unidentified vitamins. The presence of biotin, thiamine and pyridoxine in cotton batting and in Difco agar was demonstrated.

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EFFECT OF VARIATIONS IN LIGHT INTENSITY, LENGTH OF PHOTO-PERIOD, AND AVAILABILITY OF NITROGEN UPON ACCUMULATION OF ASCORBIC ACID IN COWPEA PLANTS[1]

MARY ELIZABETH REID

An understanding of the causes of variations in the ascorbic acid content of plants is desirable from both practical and theoretical considerations. Of the various environmental factors known to influence accumulation of this vitamin in plants, light appears to have the greatest effect. Differences in intensity, quality, and length of the daily period of illumination may all be concerned in producing modifying effects.

The early investigations on the effects of light upon ascorbic acid accumulation were reviewed in a previous publication (Reid 1938). Murphy (1939) has shown that the ascorbic acid content of apples is higher on the side exposed to the sun than on the shaded side. Harding, Winston, and Fisher (1939) observed significantly higher values in oranges picked from outside branches which were fully exposed to sunlight. Kessler (1939) reported that for a given variety of apple those from Southern Germany were generally richer in vitamin C than those grown in the northern sections. Fruit which matured unevenly because of shading had from 30 to 50 per cent higher ascorbic acid content on the red than on the green side. The effect was attributed to the difference in light intensity rather than to an association with the red pigment. Burrell, Brown, and Ebright (1940) found the same variety of cabbage did not have the same ascorbic acid values at different seasons or in different regions. Lojander (1940) reported considerable seasonal variation in the vitamin C content of most garden vegetables. Illynview and Ulanova (1937) found that shortening the day decreased the relative quantities of ascorbic acid in kohlrabi and sorrel and increased the content in onions. Moldtmann (1939) observed that prolonged cloudiness lowered the ascorbic acid values in plants. He also determined the vitamin C content of leaves of *Fagus sylvatica* and of *Taxus baccata* at different times of year and found the highest values in *Fagus* in July and in *Taxus* in March. Sugawara (1939) studied the influence of variations in light intensity on the accumulation of ascorbic acid in various types of etiolated seedlings. Five-day-old seedlings of radish, Chinese cabbage, and maize grown in darkness were exposed to different intensities of light for 6, 18, 30, and 42 hours at 10,000, 1,000, 100, and 10 lux and others were left in darkness. After 42 hours exposure the following values (mg./g.

[1] Publication of the figures was assisted by the Lucien M. Underwood Memorial Fund.

fresh weight) in the above sequence were found: radish, 1.11, 0.95, 0.77, 0.67, 0.61; Chinese cabbage, 1.12, 1.02, 0.79, 0.63, and 0.56; maize, 0.67, 0.63, 0.56, 0.52, and 0.49. The ascorbic acid content is thus shown to increase with increasing light intensity but not in direct proportion to it. The effect of variations in the intensity of light upon the ascorbic acid values of excised *Ficus* branches placed in water and kept in darkness for eight hours previous to assaying was studied by Moldtmann (1939). He found a 20 per cent loss during the period in darkness and a 14 per cent gain as a result of a three-hour exposure to a 40 watt light at a distance of 30 centimeters. With successive increases in light intensity up to 600 watts higher ascorbic acid values were found but as in Sugawara's results the increase in ascorbic acid was not proportional to the increase in light intensity, particularly in the higher intensities.

Some investigators who have studied the effect of soil fertility upon the vitamin C content of plants have found that high fertility is favorable to its accumulation (Potter and Overholser 1933; Ijdo 1936; Hoffman, Krauss, and Washburn 1938; Fellers, Young, Isham, and Clague 1934). Others have observed comparatively little effect (Pfützer and Pfaff 1935; Smith and Paterson 1937; Smith and Gillies 1940). High nitrogen fertilizers have generally been found to influence ascorbic acid synthesis favorably, more than do other types of fertilizers (Isgur and Fellers 1937; Balks and Pommer 1938; Sugawara 1938; Burrell, Brown, and Ebright 1940). There is little reported evidence that the use of properly balanced fertilizers decreases the ascorbic acid content of plants grown under field conditions (Virtanen 1936; Isgur and Fellers 1937). Kessler, however, found (1939) that overfertilization of apple trees with nitrogen depressed the ascorbic acid content of the fruit and suggested that it was possibly caused by the shading of the fruit with the extra-heavy foliage of the high-nitrogen trees. Fuhrmeister (1937) on the contrary had previously obtained similar results with high-nitrogen fertilization of vegetables in which shading was supposedly not an interfering factor.

Moldtmann (1939) published data suggesting a parallelism between the glucose and ascorbic acid content of leaves of monocots but not of dicots. Other experiments by this investigator showed that within certain limits increasing the available carbon dioxide resulted in an increased synthesis of ascorbic acid. On the other hand, ringing experiments, which normally lead to an increase in carbohydrates above the ring, resulted in no increase in ascorbic acid content. Leaves above a ring in *Fraxinus excelsior* had definitely less ascorbic acid than similar leaves from normal branches. No distinct difference was found in tests with *Salix capra.*

The inter-relationships of available nitrogen, carbohydrates, and growth were studied by Kraus and Kraybill (1918), Nightingale (1922), Nightingale

and Kraus (1924), Reid (1924, 1929), and by a number of other investigators. It was found that the accumulation of carbohydrates is dependent upon the quantity of available nitrogen. If the nitrogen supply is very abundant, the carbohydrates tend to be utilized in growth and in synthesis of organic nitrogen compounds. On the other hand, if the supply of nitrogen is limited, carbohydrate utilization is retarded, and a marked accumulation may result.

No investigations have been reported bearing on ascorbic acid as influenced by the interrelations of mineral nutrient supply with seasonal and weather variations in light. The present studies are concerned with some aspects of these problems.

PROCEDURE

Approximately uniform cowpea seeds of the Groit variety were placed in germinators and kept at 27–29° C. for 24 hours or slightly longer. Seedlings with sprouts approximately 1 cm. long were selected for planting in clean white sand in half-gallon crocks of the shallow type. A nutrient solution was added under the conditions indicated for individual tests.

In preparing plants for vitamin C assays, the organs were weighed rapidly, then placed in Petri dishes and kept in the refrigerator until the ascorbic acid determinations by the indophenol method could be made. The vitamin C assays were made as rapidly as possible and if there was a fairly large number of samples, tests of the leaves were always made first and of the stems last. The tissues were pulverized in a mortar using a small quantity of acid-washed sand and 5 per cent metaphosphoric acid as an extracting agent. Appropriate aliquots of the filtered extracts were used in the titrations.

EXPERIMENTAL RESULTS

1. **Effect of Seasonal Differences in Light Conditions.** Two groups of young seedlings grown without added nutrients and at different seasons of the year were assayed in the first test. Some were grown during the latter part of November under the comparatively weak illumination and short days characteristic of this season and others in the much higher light intensity and longer days of March. The records of the U. S. Weather Bureau for Washington, D. C., show that the average total amount of solar radiation per day during the period of the November test was 184 gram-calories and in the March test, 344 gram-calories. The light intensity on bright days during the November test as measured in the greenhouse by a Weston illumination meter was about 60 per cent of that on bright days in March. The plants grown during November in the weaker light had larger stems and smaller roots than those grown in March.

TABLE 1. *Comparison of total ascorbic acid values and green weights of 14-day-old cowpea plants grown in November and March*

	Nov. 20–Dec. 1 Average radiation (Gr.-cal. per day) 184		Mar. 7–17 Average radiation (Gr.-cal. per day) 344		Nov./Mar. Radiation ratio 54		
	Green Wt. G.	Asc. Ac. Mg.	Green Wt. G.	Asc. Ac. Mg.	Green Wt.	Asc. Ac. Total	Asc. Ac. Mg./G.
Leaves	0.796	0.525	0.851	0.880	0.94	0.60	0.64
Stems + Petioles	0.532	0.072	0.390	0.079	1.36	0.91	0.67
Roots	1.256	0.210	1.642	0.325	0.77	0.65	0.84
Total	2.584	0.807	2.883	1.284	0.90	0.63	0.70

Table 1 shows that the ascorbic acid values were higher in all parts of the March plants. The total ascorbic acid in the November plants was 63 per cent of that of the March plants; whereas, the total green weight of the former was only 11 per cent less than that of the latter. As calculated from the quantitative data in table 1, the leaves of the November plants contained 0.66 mg. of ascorbic acid per gram whereas those of the March plants contained 1.03 mg. per gram. The iodine and Flückiger tests[2] made upon sections of leaf and stem tissues showed a lower content of starch and free-reducing substances respectively in the November than in the March plants. The larger leaves, longer stems, smaller root systems, and lower content of starch and free-reducing substances found in these tests to be characteristic of cowpea plants grown under low light intensity and short days as compared with those grown under high illumination and long days are much like those previously described for Hubbard squash plants grown under similar conditions (Reid 1930).

In another experiment winter- and spring-cultured plants supplied with added nutrients were allowed to grow for eight weeks before being assayed. The winter group was tested on January 12 and the spring-summer group on May 19. The U. S. Weather Bureau records show that the average total radiation per day during the period in which the winter plants were grown was 165 gram-calories and during the spring-summer period was 470. The total radiation per day during the former period was only 35 per cent of that in the latter. However, during the week immediately preceding the winter test, the average total radiation per day was 41 per cent of that during the spring period. Measurements made in the greenhouse with the illumination meter showed almost a 50 per cent difference in the maximum intensities at the two seasons. The results of the experiment are shown in table 2.

[2] Eckerson, *Outlines of Plant Microchemistry* (unpublished).

TABLE 2. *Total ascorbic acid content and green weights of plants eight weeks old and tested in January and May*

	Nov. 17–Jan. 12 Average radiation 165 gr.-cal. per day		Mar. 24–May 19 Average radiation 470 gr.-cal. per day		Nov.–Jan./Mar.–May Radiation ratio 36		
	Green Wt. G.	Asc. Ac. Mg.	Green Wt. G.	Asc. Ac. Mg.	Green Wt.	Asc. Ac. Total	Asc. Ac. Mg./G.
Leaves	18.99	9.85	18.44	20.44	1.03	0.48	0.47
Stems + Petioles	15.66	1.17	13.03	1.64	1.20	0.71	0.60
Roots	9.36	1.07	17.23	2.34	0.54	0.46	0.84
Total	44.01	12.09	48.70	24.42	0.90	0.50	0.55

The winter plants accumulated half as much ascorbic acid as the spring-summer plants. The relative difference in size of the root systems is greater than that observed in the younger plants of the first test. A pronounced difference in the total and percentage ascorbic acid values and only a slight variation in green weight in the leaves of the two groups of plants is to be noted.

Some of the plants in the November-December test were grown under a cheesecloth screen which reduced the light intensity about 50 per cent. On January 12, when the plants were 8 weeks old, tests were made to determine the ascorbic acid values of successive leaves of the shaded and unshaded plants. The data thus obtained are shown in table 3 together with the results of similar assays of the leaves of the plants tested on May 19.

The leaves of the winter plants contained 57 per cent as much ascorbic acid expressed as absolute amount and 48 per cent as much on a green

TABLE 3. *Ascorbic acid content of leaves of plants grown in normal and reduced intensity of daylight and at different seasons*

	Eight-week-old plants tested Jan. 12				Eight-week-old plants tested May 19	
	Plants grown in daylight of normal intensity		Plants grown in partial shade			
	Mg./Plt.	Mg./G.	Mg./Plt.	Mg./G.	Mg./Plt.	Mg./G.
First (oldest) leaf	0.47	0.46	0.30	0.39	0.43	0.91
Second " "	0.66	0.43	0.35	0.30	1.21	1.02
Third " "	1.11	0.47	0.51	0.32	1.99	1.04
Fourth " "	1.49	0.47	0.77	0.38	3.21	1.07
Fifth " "	1.69	0.51	0.93	0.38	3.26	1.10
Sixth " "	1.67	0.47	0.95	0.38	2.75	1.04
Seventh " "	1.60	0.49	0.97	0.37	2.86	1.16
Eighth " "	1.43	0.68	1.04	0.48	2.32	1.19
Ninth " "	0.83	0.94	0.55	0.63	1.10	1.66
Total	10.94	0.52	6.37	0.39	19.13	1.09

weight basis as did those of the spring-summer plants. Shading of the winter plants caused a reduction of approximately 42 per cent in total ascorbic acid and a 25 per cent lower value on a percentage green weight basis.

2. Effect of Varying Intensities of Light. Further studies were conducted to determine the effect of varying light caused by changing weather conditions on ascorbic acid accumulation. Twelve-day-old plants grown in normal daylight were assayed for ascorbic acid on April 4 at 2:00 p.m. At approximately the same time on the next day cultures from the following environments were assayed: normal light intensity, normal light reduced about 75 per cent by a cheesecloth screen, such partial shade cultures which had been transferred to full daylight 24 hours previously, and normal daylight cultures transferred to darkness 20 hours previously. As indicated in table 4, approximately similar procedures were followed on the next two days.

The plants grown under reduced light intensity contained only 40 per cent as much ascorbic acid on the basis of absolute amount as those grown in normal light and 59 per cent as much on a green weight basis. The greatest effect of the difference in light intensity was found in the leaves and least in the stems. Exposure of the shaded plants to normal daylight for twenty-four hours caused an increase in the total ascorbic acid, most of the increase being in the leaves. Exposure to normal daylight for two days following partial shading resulted in a 55 per cent higher value for the absolute amount than was found in similar plants left in the shade. At this time a 20 per cent higher total value in the roots and a 24 per cent higher total value in the stems was found in the more highly illuminated plants. Keeping the plants in darkness for twenty-four hours previous to assaying resulted in losses of the vitamin of approximately 15 per cent. The data indicate that all parts of the plant were involved in the decrease. Plants grown in daylight of normal intensity and transferred to the low intensity chamber forty-eight hours before assaying had an 11 per cent lower total ascorbic acid value on April 6 and others a 24 per cent lower value on April 7 than similar plants left in normal daylight. The lower value of the normal plants on April 6 was associated with very cloudy weather. Presumably at the time of assaying complete recovery from the metabolic loss of the previous night had not occurred because of the weak light (Reid 1941).

3. Comparative Effects of Differences in Light Intensity and Length of Day with and without Additional Nitrogen. An experiment was conducted in March in which environmental variations were employed, simulating in some respects the normal seasonal changes in light intensity and length of daily period of illumination. Thirty-six cultures of ten plants each were prepared, nine of which were placed under each of the four following experimental conditions:

TABLE 4. *Variations in growth and ascorbic acid content of young cowpea plants in relation to variations in conditions of illumination*

Conditions during growth		Green Wt. (g.)	Ascorbic Acid (mg.)	Ascorbic Acid (mg./g.)
		April 4—Very bright		
12 days normal daylight	Entire Plant	2.56	1.070	0.418
	Stems	0.37	0.081	0.215
	Roots	1.52	0.293	0.193
	Leaves	0.675	0.696	1.031
		April 5—Very bright		
13 days normal daylight	Entire Plant	2.78	1.280	0.460
	Stems	0.37	0.101	0.277
	Roots	1.68	0.347	0.253
	Leaves	0.728	0.832	1.143
12 days normal daylight, 1 day in darkness	Entire Plant	2.58	0.904	0.346
	Stems	0.37	0.069	0.187
	Roots	1.51	0.245	0.162
	Leaves	0.703	0.590	0.839
13 days weak daylight	Entire Plant	1.88	0.510	0.271
	Stems	0.45	0.068	0.151
	Roots	0.83	0.156	0.187
	Leaves	0.596	0.286	0.480
12 days weak daylight, 1 day normal daylight	Entire Plant	1.97	0.570	0.289
	Stems	0.49	0.073	0.150
	Roots	0.84	0.158	0.188
	Leaves	0.640	0.339	0.530
		April 6—Very cloudy		
14 days normal daylight	Entire Plant	2.66	1.079	0.406
	Stems	0.38	0.095	0.245
	Roots	1.60	0.314	0.196
	Leaves	0.678	0.670	0.988
12 days normal daylight, 2 days weak daylight	Entire Plant	2.79	0.818	0.258
	Stems	0.40	0.077	0.193
	Roots	1.70	0.291	0.171
	Leaves	0.692	0.450	0.650
14 days weak daylight	Entire Plant	2.24	0.456	0.203
	Stems	0.54	0.066	0.122
	Roots	1.00	0.169	0.168
	Leaves	0.696	0.222	0.319
12 days weak light, 2 days normal daylight	Entire Plant	2.21	0.710	0.322
	Stems	0.55	0.082	0.148
	Roots	1.00	0.203	0.203
	Leaves	0.664	0.425	0.640

TABLE 4—(*Continued*)

Conditions during growth		Green Wt. (g.)	Ascorbic Acid (mg.)	Ascorbic Acid (mg./g.)
		April 7—Very bright		
15 days normal daylight	Entire Plant	2.92	1.104	0.365
	Stems	0.41	0.099	0.241
	Roots	1.81	0.345	0.191
	Leaves	0.700	0.660	0.943
15 days weak daylight	Entire Plant	2.39	0.533	0.223
	Stems	0.55	0.068	0.124
	Roots	1.16	0.175	0.155
	Leaves	0.678	0.290	0.428
13 days normal daylight, 2 days weak daylight	Entire Plant	2.90	0.978	0.339
	Stems	0.41	0.108	0.263
	Roots	1.82	0.307	0.168
	Leaves	0.676	0.563	0.833
14 days normal daylight, 1 day darkness	Entire Plant	2.75	0.924	0.336
	Stems	0.38	0.081	0.213
	Roots	1.69	0.300	0.176
	Leaves	0.676	0.543	0.803

[a] Last day was very cloudy.

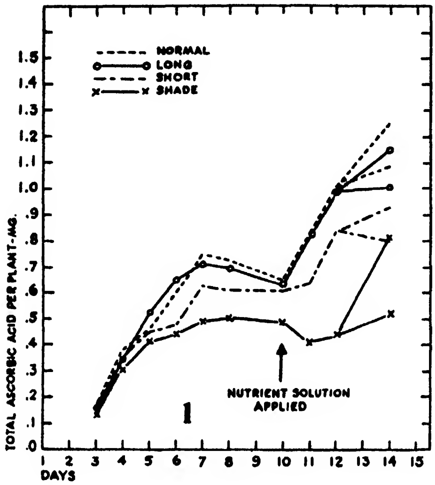
1. Normal light intensity and normal length of day.
2. Short day. Daily illumination period of seven hours. Frame covered with black sateen placed over plants at 4:00 p.m. and removed at 9 a.m.
3. Long Day. Daily illumination period of seventeen hours. 150 watt Mazda lights placed 30 inches above the plants were on from 5:00 to 11:00 p.m.
4. Partial shade. Plants were placed under a frame covered with cheesecloth which reduced the normal light intensity in the greenhouse by about 65 per cent.

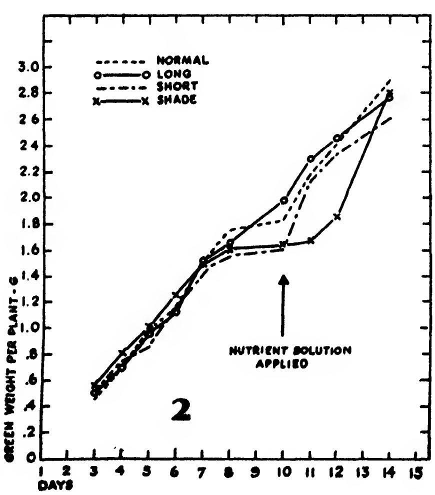
On the tenth day and each day thereafter a complete nutrient[3] solution was applied. The ascorbic acid assays were begun on the third day when the primary leaves were emerging above the surface of the sand. Roots, stems, and leaves were analyzed separately. Leaf areas were also determined. Plants grown under the different environmental conditions were prepared for the tests at 11:00 a.m.

Reducing the light intensity during the month of March had a much more marked effect upon ascorbic acid values than did lengthening or shortening the daily period of illumination. During the time of partial dependence of the seedlings upon the stored food reserves in the cotyledons,

[3] The mineral elements were present in the following concentrations (p.p.m.): N = 90, P = 45, K = 75, Ca = 40, Mg = 20, S = 36, Cl = 58, Fe = 2.5, Mn = 0.1, B = 0.1, Zn = 0.1.

that is up to and including the fifth day, variations in light intensity or in length of the daily period of illumination had less influence than after the plants became more nearly independent. The total ascorbic acid per plant at successive stages of development under each of the four environmental conditions is shown in figure 1 and the green weights of the plants in figure 2.





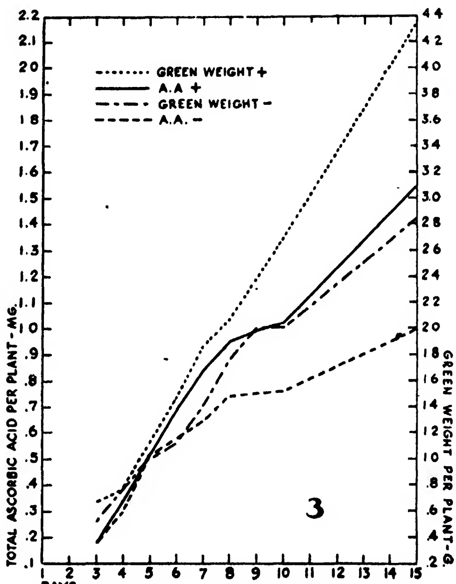


FIG. 1. Total ascorbic acid content of young cowpea plants grown under different conditions of illumination. The lower points on the curve on the fourteenth day represent the ascorbic acid values exclusive of the compound leaves and the upper points the values with the compound leaves included. FIG. 2. Green weights of young cowpea plants grown under different conditions of illumination. FIG. 3. Green weights and total ascorbic acid in young plants, some grown with (+) and some without (−) added nutrients.

From the seventh to the tenth days inclusive there was a lag in ascorbic acid accumulation under each of the four conditions of illumination. There was also a decrease in the rate of growth apparent on the seventh or eighth days, continuing to the tenth day. A pronounced lag in growth and ascorbic acid accumulation at this stage of development has also been noted in previous experiments in plants grown without added nutrients and a slight retardation in ascorbic acid increase even in well fertilized plants (Reid 1941).

The (total) ascorbic acid of the short-day and partially shaded plants remained practically constant from the seventh to the tenth days; whereas, the results suggest a loss from the normal and long-day plants on the tenth day (Reid 1941). No determinations were made on the ninth day. The apparent losses followed two days of very cloudy weather. There is a suggestion here, as there also has been in previous tests, that metabolic losses of ascorbic acid during periods of darkness are greatest in plants which have been most active photosynthetically during the immediately preceding periods of illumination.

Microchemical examination of the tissues revealed that the normal- and long-day plants had accumulated considerable starch and free-reducing substances, those of the short-day plants somewhat less, and those of the shaded plants relatively little. The internal symptoms of the normal- and long-day plants suggested that a state of nitrogen deficiency was developing. On the eleventh day, following the application of nutrient solution on the tenth day, a definitely higher ascorbic acid content and a stimulation of growth were found in these plants. An appreciable gain in ascorbic acid was not evident until a day later in the short-day plants. The addition of nutrients appeared to cause a depression in the total amount of ascorbic acid in the shaded plants for two days immediately following application of the solution. Between the twelfth and fourteenth days, however, a very rapid gain occurred. This was caused chiefly by the rapid development of the first set of compound leaves. The ascorbic acid values on the fourteenth day, with and without inclusion of the compound leaves, may be observed in figure 1, the lower points on the fourteenth day representing the values without the compound leaves and the upper points the values with the compound leaves included. A more rapid development of the first set of compound leaves was observed in the shaded plants than in the other groups. This tendency toward a somewhat more rapid initiation and development of leaves has been noted previously in plants grown in light but under conditions allowing a relatively slow rate of carbohydrate synthesis (Reid 1930).

In other tests conducted at a time of year when light conditions were similar to those of the preceding test no nutrients were added but nodules were allowed to develop on the roots. The apparent availability of nitrogen

was soon reflected in an increase both in growth and in total ascorbic acid similar to the responses to nutrient solution. The results of one of these tests are shown in figure 3. The plants which had received nutrient solution since the third day (the time of emergence above the sand) were much larger on the fifteenth day than the plants which received no nutrient solution. No assays were made between the tenth and fifteenth days. Doubtless, if daily determinations of ascorbic acid in the plants grown without added nutrients had been made during this period, the curve would have continued in the horizontal direction for some time after the tenth day and, as nitrogen became available, would then have risen more steeply than is represented in the graph. The results of this experiment suggest that the stimulatory effect on growth and ascorbic acid synthesis noted after application of a complete nutrient solution to very young plants is caused chiefly by the added nitrogen. The addition of nutrient solution lacking in other elements[4] such as phosphorus, potassium, calcium, and magnesium have not produced definite differences in the total ascorbic acid in plants at such an early stage of development. Apparently these elements become limiting factors both in growth and in ascorbic acid synthesis at later stages than does nitrogen.

Reducing the light intensity resulted in a greater spread of the leaf blade which became evident as early as the fifth day, later becoming more pronounced. There were, however, no marked differences in the green weights of leaves of the four groups of plants. The greater thickness of the leaves of the plants grown in normal daylight as compared with the leaves of the shaded plants compensated in weight for the smaller areas of blades of the former. On the fourteenth day the primary leaves of plants grown in daylight of normal intensity both under normal- and long-day conditions contained 0.019 mg. of ascorbic acid per square centimeter of leaf surface whereas those of the shaded plants contained only 0.007 mg. per square centimeter and those of the short-day plants 0.016 mg. The thicker leaves of the plants grown in daylight of normal intensity contained a larger number of chloroplasts per unit area of tissue than the thin leaves of the shaded plants, a difference which undoubtedly has a determining influence upon the ascorbic acid concentration per unit area of mesophyll surface. There is considerable evidence that ascorbic acid synthesis is associated with the functioning of the chloroplasts (Geitler 1922; Girond, Leblond, and Ratsimanga 1934; Gautheret 1935; Wieler 1936; Weber 1936; Dischendorfer 1937; Pekarek 1938; Weier 1938; Bukatsch 1940). Tests made with very young cowpea leaves, before there is much development of the mesophyll, have shown that the ascorbic acid content is about the same as that of the young portion of the stem; but with development of the mesophyll under favorable light conditions there is an increase of several hundred per cent

[4] Unpublished data.

per unit of weight. For example, young leaves from the seventh node which were enclosed within bud scales but with tips emerging yielded values of 0.22 mg. per gram; whereas those at the sixth node which were expanding after emergence from the bud scales contained 1.31 mg. per gram.

The partially shaded plants of the present experiment had longer, heavier stems than those of the other three groups grown in daylight of normal intensity, but the ascorbic acid values tended to be somewhat lower, the differences increasing as the plants became older. Toward the end of the experimental period, when the first set of compound leaves was developing, the differences were pronounced. Somewhat lower values were observable also in the short-day as compared with the normal and long-day plants in the latter portion of the test period.

The ascorbic acid values for the roots of the different groups of plants varied in the same directions as did those of the stems, low intensity being the only one of the four conditions causing marked differences. For example, the roots of normal-, short-, and long-day plants respectively contained 0.34, 0.25, and 0.31 mg. of ascorbic acid on the fourteenth day, whereas those of the shaded plants contained only 0.13 mg.

TABLE 5. *Summary of results in the preceding experiments on the relation of light intensity to ascorbid acid accumulation*

Maximum Light Intensities (approximate values in f. c.)	Experiment	1	2	3	4	5
2500	Total (Mg. per plant)				0.510	
	Mg./G. in leaves			0.390	0.403	
3500	Total (Mg. per plant)					0.420[a]
	Mg./G. in leaves					0.480
5000	Total (Mg. per plant)					
	Mg./G. in leaves		0.519	0.520		
6000	Total (Mg. per plant)	0.807				
	Mg./G. in leaves	0.660				
9000–10,000	Total (Mg. per plant)	1.284			1.280	1.090[a]
	Mg./G. in leaves	1.034	1.108	1.090	1.015	1.143

[a] These values are somewhat low, because the nutrient solution was not added until the plants were ten days old. Total values in experiments 2 and 3 cannot be compared with the others because the plants were much older.

Table 5 shows the ascorbic acid content (mg./g.) of the leaves of the plants employed in the previous experiments for which different light intensities prevailed. The values for the latter represent approximately the maximum intensities found during the course of the experiments. A comparison of the total quantity of vitamin C of plants grown under different light intensities cannot be made in experiments 2 and 3 because the plants were eight weeks old as compared with fourteen- and fifteen-day-old plants in the other three tests. A relation between light intensity and both absolute and percentage content of ascorbic acid is clearly indicated but further tests using more refined methods are required to determine exact quantitative relationships.

DISCUSSION

The data herein presented afford an insight into some of the more common causes of variation in the vitamin C content of green plants. The differences in light factors characteristic of different seasonal and weather conditions are of importance. It should be emphasized that these results were obtained chiefly with young seedlings which are known to be very sensitive to differences in light intensity. It is quite possible that the length of day factor would have a greater influence in older cowpea plants or in other types of plants. An investigation of the ascorbic acid relations in plants whose reproductive period is determined by length of day would be of special interest. It is recognized that reducing the average light intensity of plants grown in March, April, and May to approximate the average intensity in December by the methods herein employed afford at best only a crude approach toward natural climatic conditions.

The present results are in general agreement with those of Sugawara and Moldtmann in showing that the ascorbic acid content of plants increases with increasing light intensity and they also suggest that the relationship may not be linear. Sugawara and Moldtmann have shown that differences in illumination at the lower intensities produce relatively greater differences in ascorbic acid synthesis than similar differences in the higher intensities. This is also the now generally accepted view of the relation between variations in light intensity and the rate of carbohydrate synthesis.

The question of the relative importance of light intensity and the total amount of light in influencing ascorbic acid synthesis is still unsettled. Light intensity is of great importance, in part, because of its effect upon the structural development of the leaf with respect to the organization and depth of the palisade tissue, thereby affecting the number and distribution of the chloroplasts. It may also be essential for the synthesis of a protein-like constituent contained within the latter. However, we do not yet know whether its chief importance consists in the formation of these structural elements or whether it is mainly concerned in their functioning. If it is

important in connection with the former, then a time factor in ascorbic acid synthesis may be of major importance. For example, an etiolated seedling placed in darkness as in Sugawara's experiments would tend to function slowly in ascorbic acid synthesis until an increase occurred in some other constituent, supposedly originating in the chloroplasts.

The results of the present tests and those of other workers suggest the possibility of a functional relationship between the synthesis of ascorbic acid and that of carbohydrates. It is possible that the product of one of these two types of synthesis may result from that of the other but the question remains as to which, if either, takes precedence. It is possible, on the other hand, that these substances may be simultaneous products of chloroplastic activity.

Previous studies (Reid 1930, II) have shown the importance of light and the necessity of a supply of nitrogen for the synthesis and maintenance of a plasmatic constituent of the chloroplasts. Since evidence suggests that the chloroplasts are the structures mainly concerned in the formation of ascorbic acid, an increased rate of synthesis when nitrogen is supplied to nitrogen-depleted plants is not surprising.

The data suggest that if a plant is grown under light conditions which permit a rapid synthesis of carbohydrates, ascorbic acid synthesis will be rapid also. If there is a deficiency of nitrogen, carbohydrates tend to accumulate, not so much because more are produced as because utilization is retarded. Under these conditions ascorbic acid may increase very slowly at first, and then will cease to accumulate as the deficiency becomes more pronounced. The evidence so far is inconclusive as to whether an abundance of nitrogen during periods of limited illumination is a depressing factor for the content of ascorbic acid as it is known to be for carbohydrates. At least it seems clear that high soil fertility with reference to nitrogen is conducive to vitamin C production only when light conditions allow synthesis of an ample supply of carbohydrates. On the basis of Fuhrmeister's (1937) and Kessler's (1939) work a very high nitrogen supply may actually be detrimental. Further study is required to determine conclusively to what extent such harmful effects may be overcome by increasing the amount of light.

These results suggest that weather conditions should be considered in determining the time for harvesting fruits and vegetables. Other experiments which will be described in detail elsewhere have shown that time of day is also important, especially for vegetables of leafy types. Consideration of these factors may be of special value in timing the selection of material for canning.

SUMMARY

High daylight intensity and medium to long days are conducive both to high absolute and high percentage ascorbic acid values in cowpea plants; whereas low intensity and short days have a depressing effect.

Seasonal variations, attributable to differences in conditions of illumination were found in the absolute and percentage content of ascorbic acid.

With differences in light intensity of 65 per cent and in length of the illumination period with normal daylight of 42 per cent, light intensity was found to have a greater influence than length of day. Lengthening the day with weak light (Mazda) to 17 hours so as to produce an 80 per cent increase in day length did not produce increases as great as a 65 per cent increase in light intensity.

Shifting ascorbic acid values were found as a result of environmental alterations such as occur under changing weather conditions. The variations are noted first in the leaves and somewhat later in the roots.

A sufficient supply of available nutrients with special reference to nitrogen is also conducive to high ascorbic acid values, particularly as to the total quantity, provided light conditions are such as to permit the synthesis of an ample supply of carbohydrates. It is suggested that the special relation of nitrogen to ascorbic acid formation in a green plant is dependent upon the necessity of this element for the synthesis and maintenance of the plasma content of the chloroplasts.

It is suggested that weather conditions involving varying intensities of light should be considered in determining the time for harvesting fruits and vegetables.

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THE RELATION BETWEEN XYLEM THICKENINGS IN PRIMARY ROOTS OF VICIA FABA SEEDLINGS AND ELONGATION, AS SHOWN BY SOFT X-RAY IRRADIATION

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Previous studies on the root structure of *Vicia faba* seedlings grown from x-rayed seeds reported modifications in the secondary wall thickenings of the xylem elements (6). One observation indicated an alteration in the normal sequence of secondary wall thickenings including the annular, spiral, scalariform and pitted types. In the roots of these treated seedlings, very prominent pitted vessels occurred slightly above the apical meristem with only an occasional spiral or scalariform element in some specimens.

Morphology texts such as those of Jeffrey (2) and Haberlandt (1) discuss the relationship between these vessel types and the extent of elongation in the stem or root. A more complete study of this point is given in an early work of Stover (7) on the vascular anatomy of *Calamovilfa longifolia.* In it he maintains that the vessel types are the result of elongation. His observations indicate that annular and spiral xylem elements, which are rarely present in the rhizome of *Calamovilfa* because of the failure of internodal elongation, are present in the rapidly elongating aerial stems, as are also the later formed reticulate and pitted elements.

It is further implied by Stover, that the first thickening of a desmogen cell is of the pitted type, being laid down in the arrangement of a pitted cytoplasmic cell lining, the latter being a result of a cellular vacuolation which occurs early in the development of young cells of desmogen strands. After the formation of the original pitted thickening, division and elongation of surrounding parenchymatous cells causes it to be torn apart and to form annular, spiral, scalariform, or pitted arrangements, depending upon the extent of stretching. Consequently a pitted vessel of the metaxylem remains as such if the surrounding cells are no longer dividing or enlarging.

If the extent of elongation, therefore, determines the type of wall thickening of the xylem elements, variations in both of these factors in the roots of seedlings germinated from x-rayed seeds ought to show some relationship. On this basis, together with the fact that, within limits, the extent of elongation of the primary roots of plants grown from x-ray treated seeds decreases as the dose of irradiation increases, it seemed likely that a study of a series of *Vicia faba* primary roots with successively less elongation correlated with increasing x-ray irradiation might contribute information on

[1] The cost of publication of the figures was borne in part by the Department of Physics of the University of Cincinnati.

the relationship existing between the types of vessel secondary wall thickening and total elongation.

METHODS

The soft x-ray apparatus used here and the general character of its radiation have been described previously (3, 4). Dry *Vicia faba* seeds were placed with their hila toward the source of radiation in small glass dishes 8 cm. from the focal spot of the x-ray tube. The tube was operated at 30 peak kv., and 10 ma. Exposure times included 0, 15, 30, 60, 90, and 120 minutes.

FIG. 1. Decreasing root lengths of 5-day-old *Vicia faba* seedlings grown from seeds x-ray irradiated at 30 kv., and 10 ma. for 0, 15, 30, 60, 90, and 120 minutes.

After irradiation, the seeds were soaked for 5 hours and later germinated in moist peat moss at 25° C. Material for histological studies was collected after five days, when the seedlings germinated from the seeds exposed for 120 minutes displayed the characteristic cessation of growth associated with the "delayed killing" effect in seedlings from x-ray irradiated seeds, as described by Maxwell (5). Primary roots of 50 seedlings of each experimental lot were prepared for microscopical study with standard methods of microtechnique.

In studying the histological developments in seedlings grown from x-ray treated seeds, it is necessary to prepare and observe a great quantity of sections because of the resulting irregularity in arrangement and formation

of tissues. Consequently the longitudinal sections used as illustrations in this paper are not medial throughout their entire extent, but they approach this position as nearly as possible, and the developments indicated represent data from several hundred slides.

OBSERVATIONS

Figure 1 indicates the extent of primary root elongation in seedlings grown from seeds which had been exposed to soft x-rays for periods of 0, 15, 30, 60, 90, and 120 minutes. The characteristic decrease in root length with increasing doses of x-ray irradiation is shown. The average primary root lengths, including the hypocotyl, of seedlings of each lot, are listed in Table 1.

TABLE 1

	Time of exposure to x-rays in minutes					
	0	15	30	60	90	120
Average length of primary root in centimeters	8.0	5.8	5.0	4.0	3.6	3.0

The failure of lateral root formation related to x-ray treatments of seeds is also observed. A discussion of this has already been given (6).

Medial longitudinal sections of the root tips of these seedlings, showing types of secondary wall thickenings of the xylem elements, are illustrated in figures 2–7. Figure 2 shows the occurrence of the vessel elements and the order of their formation in normal primary roots of *Vicia faba* seedlings. The relative range of prominence of each vessel type, as it appears in the roots of the seedling groups studied, is diagrammed in figure 8.

In examining longitudinal root sections of the seedlings germinated from x-ray irradiated seeds, the most evident modification of the normal arrangement is the appearance of large pitted vessels in the most distal part; vessels of other types are found in the earlier formed regions above (figs. 3–7). If pitted vessels are associated with the least elongation of the structure in which they are forming, as is suggested by Stover, and if types formed before these indicate more extensive elongation, one is led to assume that the appearance of elements related to least elongation during later development indicates a decline in growth so far as elongation is involved.

This implication becomes plausible when one considers the phenomenon of "delayed killing" or growth cessation (5) which occurs in seedlings from seeds given a medium dose of x-rays. After an apparently normal onset of germination, inasmuch as the radicles appear beyond the cotyledons at approximately the same time in both control and treated seedlings; there is a progressive decline in rate of elongation resulting ultimately in death. The

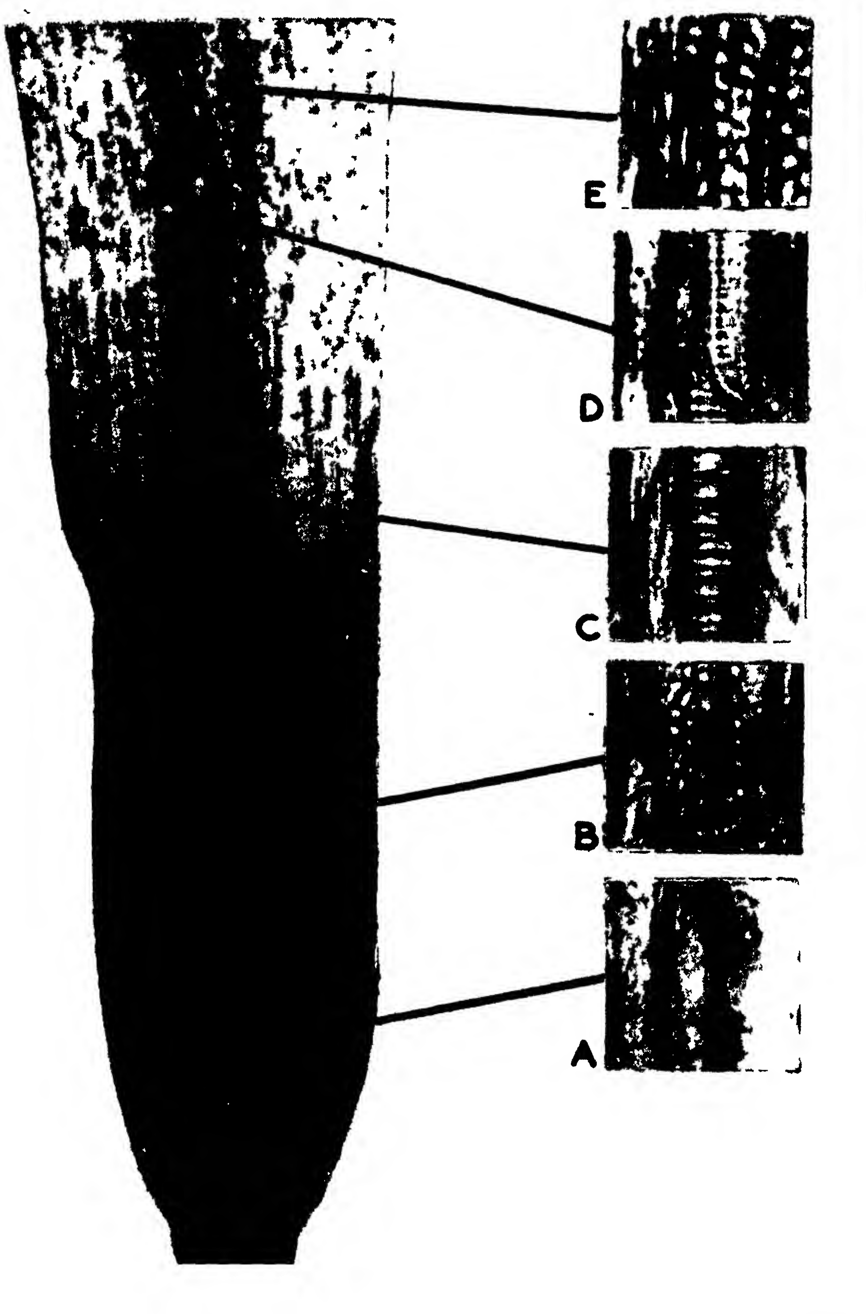

FIG. 2. Medial longitudinal root tip section of a normal *Vicia faba* seedling showing the occurrence of vessel types. × 40 and 675.

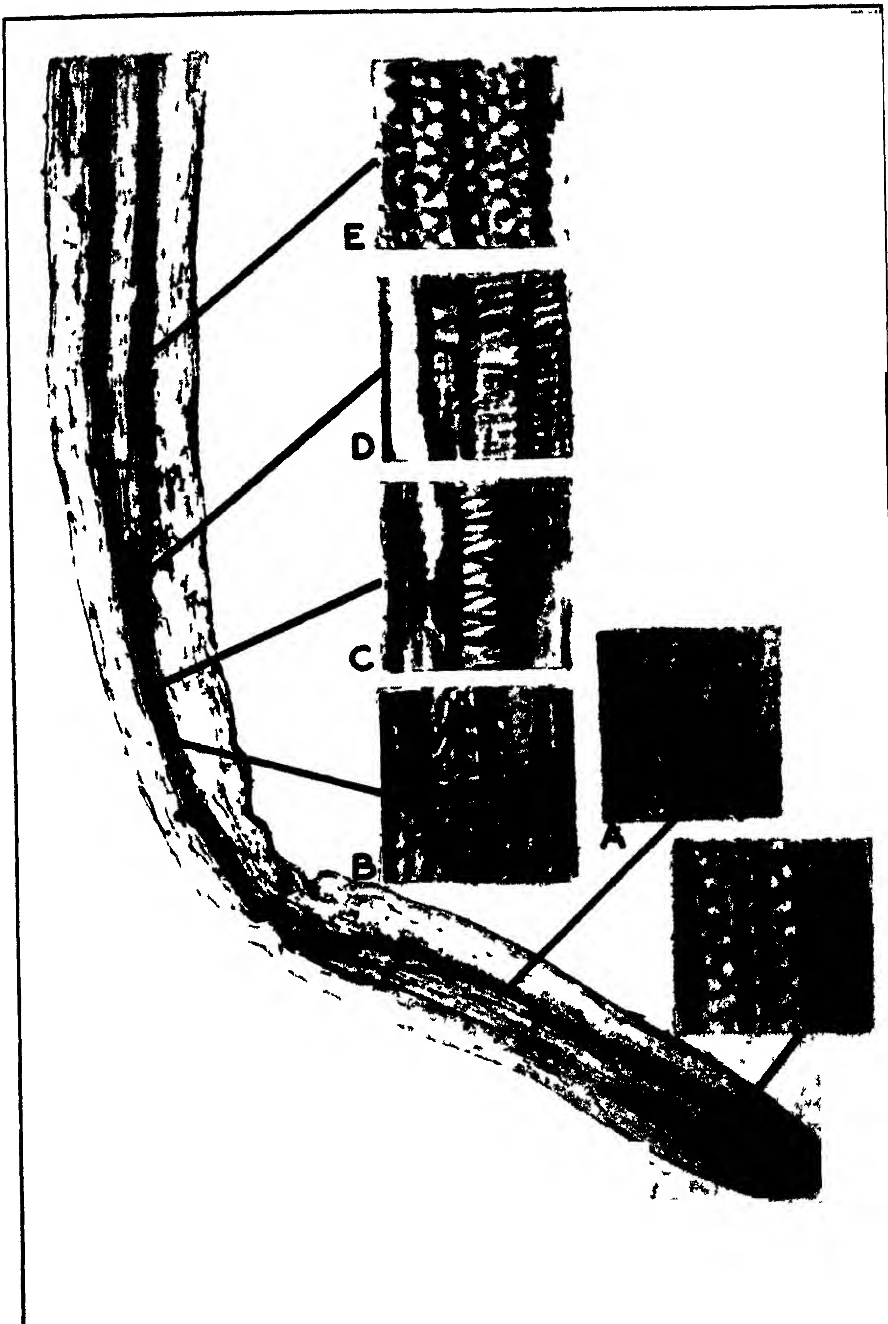

FIG. 3. Medial longitudinal root tip section of a *Vicia faba* seedling grown from a seed x ray irradiated at 30 kv., and 10 ma. for 15 minutes. × 20 and 675.

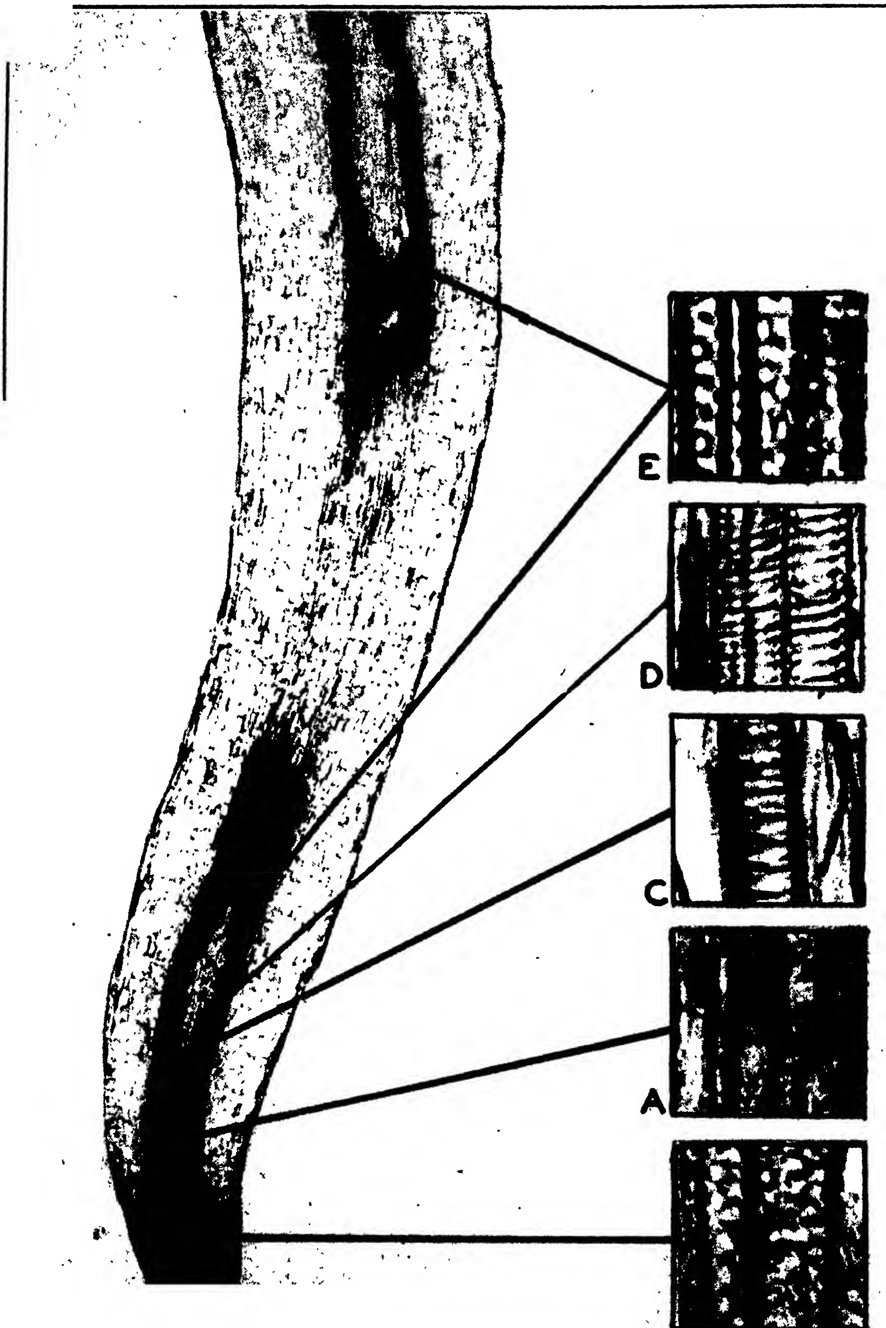

FIG. 4. Medial longitudinal root tip section of a *Vicia faba* seedling grown from a seed x-ray irradiated at 30 kv., and 10 ma. for 30 minutes. × 20 and 675.

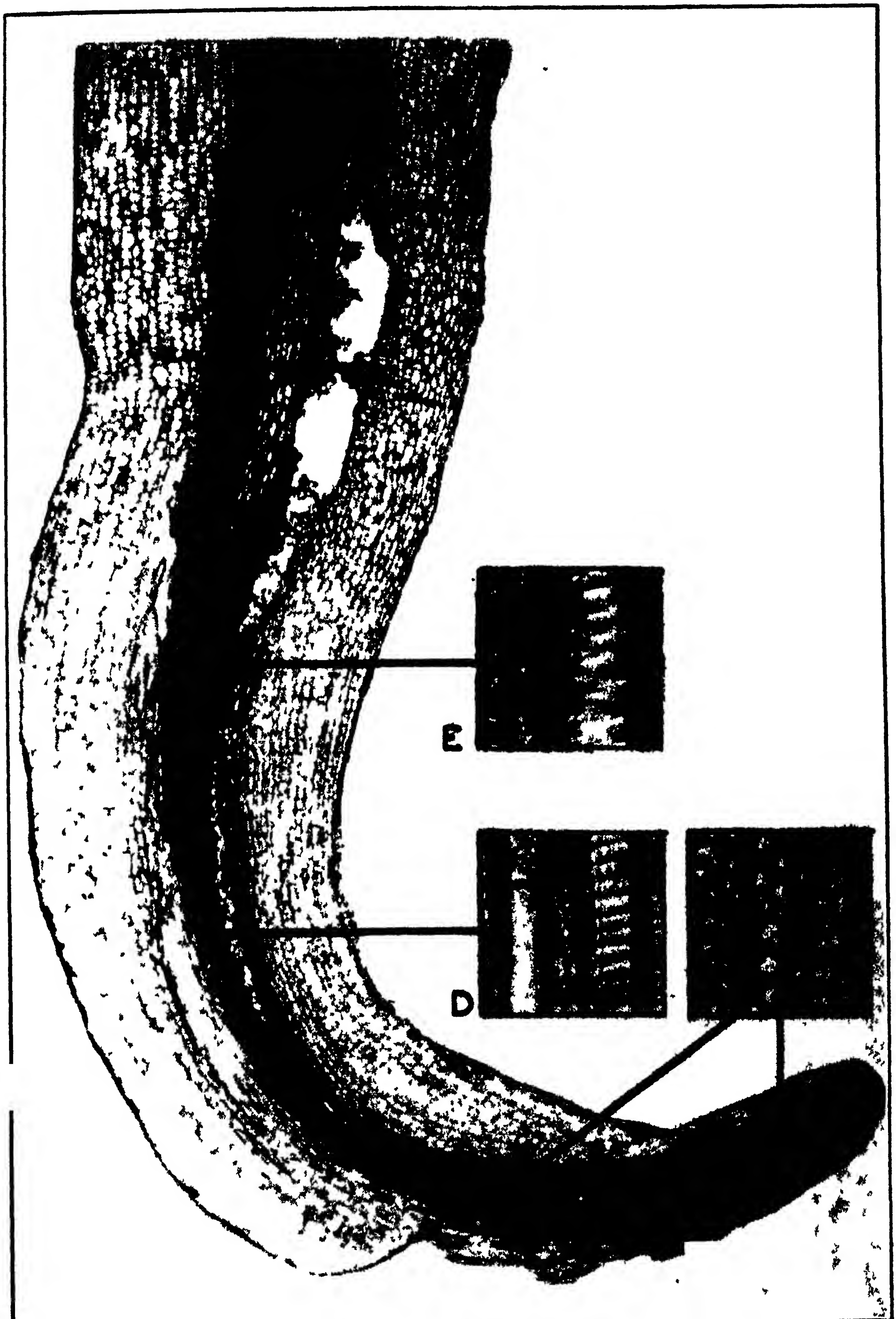

FIG. 5. Medial longitudinal root section of a *Vicia faba* seedling grown from a seed x-ray irradiated at 30 kv., and 10 ma. for 60 minutes. × 20 and 675.

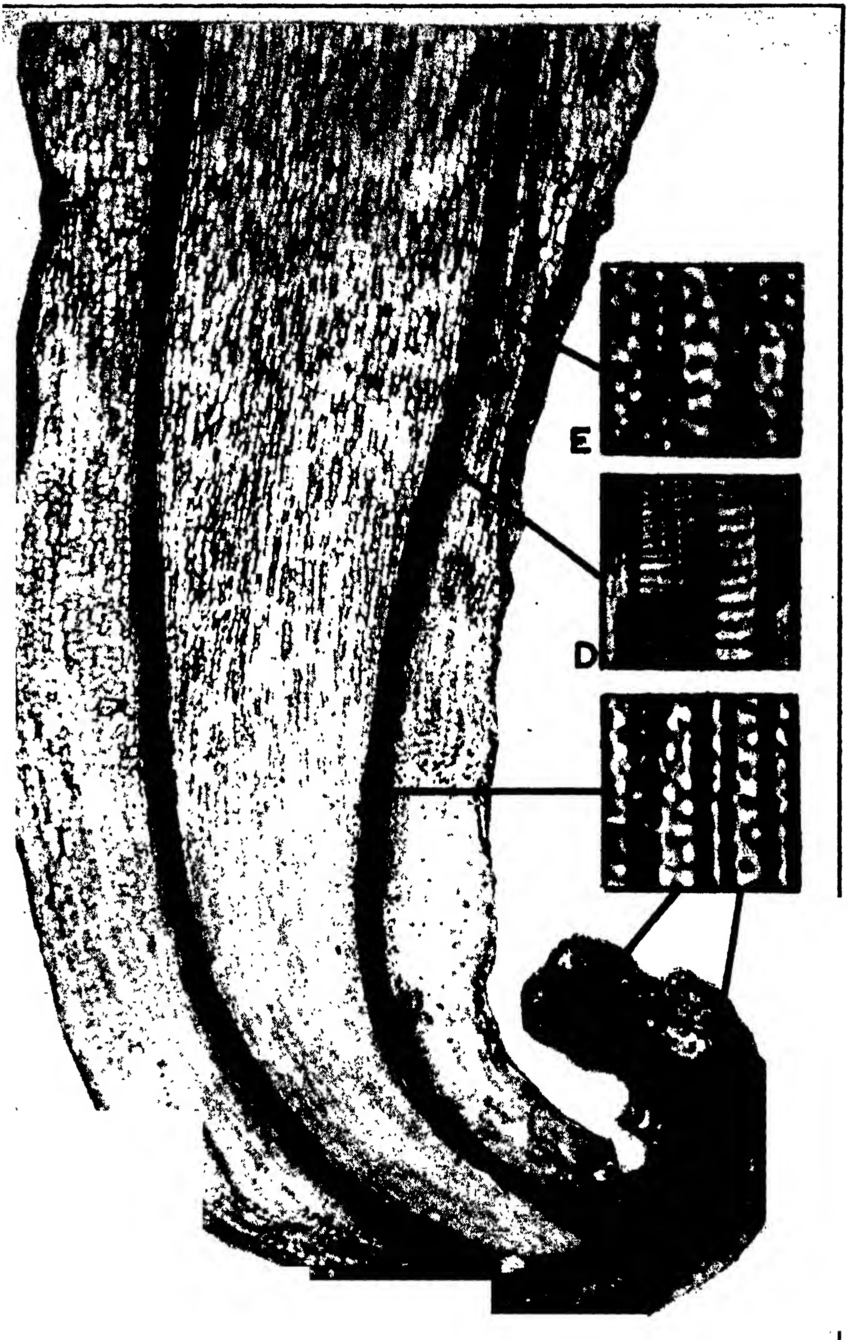

FIG. 6. Medial longitudinal root tip section of a *Vicia faba* seedling grown from a seed x-ray irradiated at 30 kv., and 10 ma. for 90 minutes. × 20 and 675.

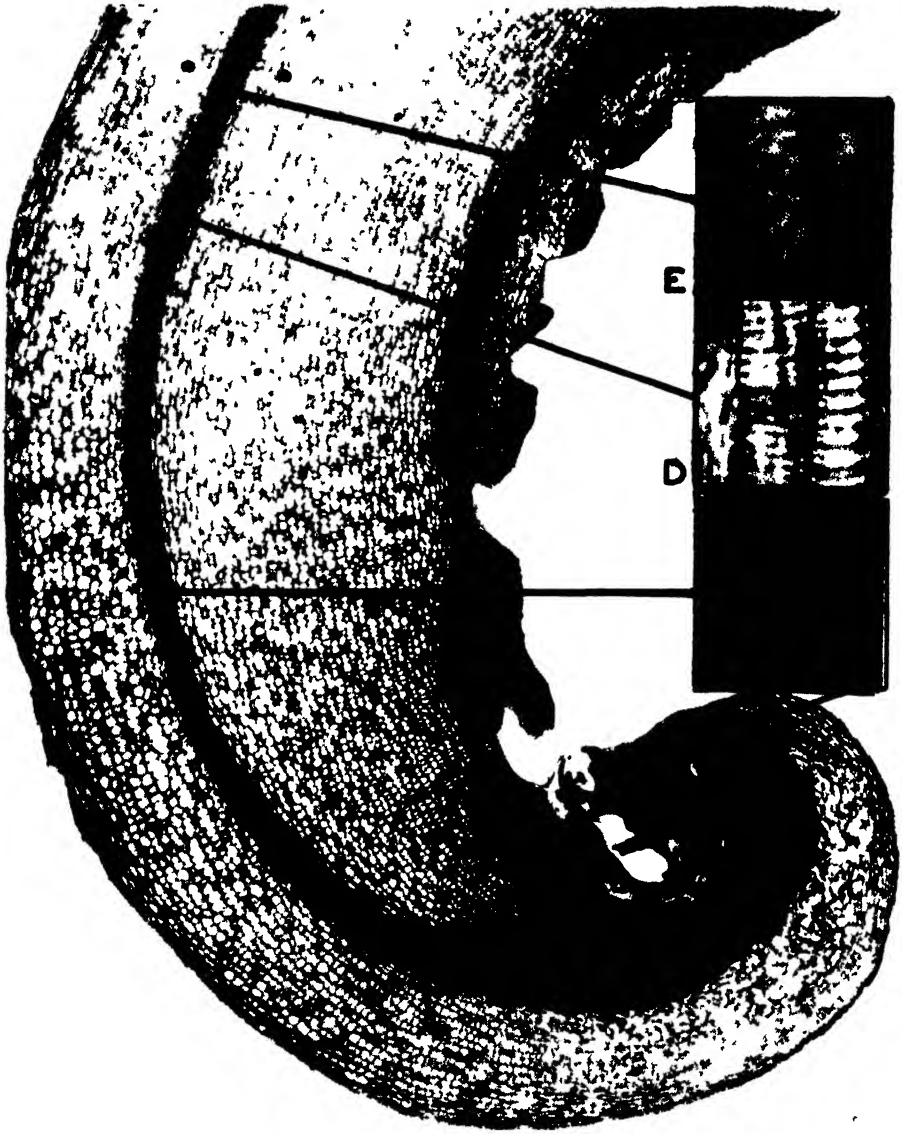

FIG. 7. Medial longitudinal root tip section of a *Vicia faba* seedling grown from a seed x-ray irradiated at 30 kv., and 10 ma. for 120 minutes. × 20 and 675.

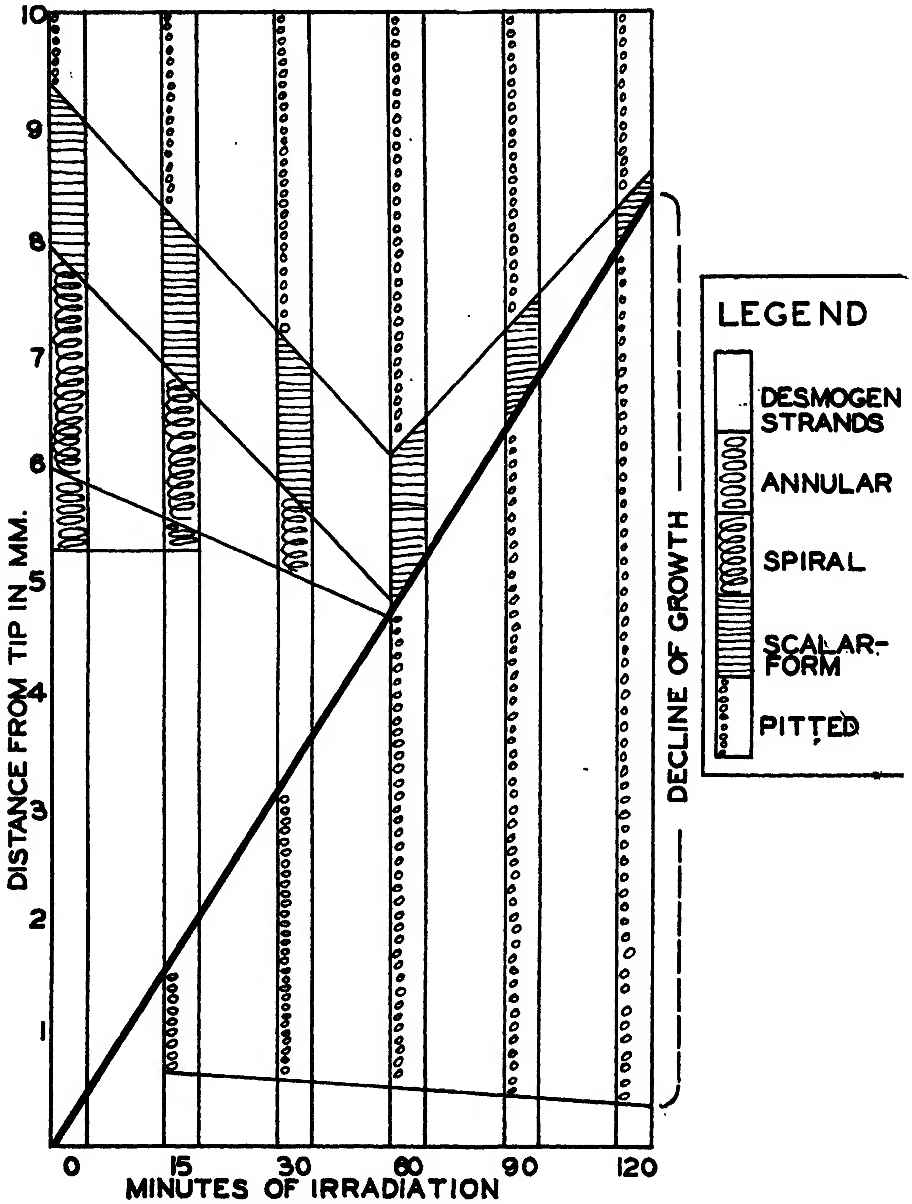

FIG. 8. Diagram showing the order and relative range of vessel types in the root tips of control *Vicia faba* seedlings and of seedlings grown from seeds x-ray irradiated at 30 kv., and 10 ma. for 15, 30, 60, 90, and 120 minutes.

total extent of elongation and the length of their germination period before subsequent decline are dependent upon the magnitude of the x-ray treatment. They are found to decrease with increasing treatments. It seems justi-

fiable to proceed further and to associate histological data with these external observations.

It would be expected that indications of decline in extent of cellular elongation in the roots would appear first in the most recently formed vessels. This is observed in all seedlings from the x-ray treated seeds; in them, portions of the root tip have become completely permanent in aspect by the increasing presence of xylem elements with pitted secondary wall thickenings. The effect is found to become more pronounced with higher x-ray treatments. It is very prominent in the root tip sections of seedlings grown from seeds irradiated for 60, 90 and 120 minutes (figs. 5, 6, 7). It seems quite possible, therefore, that there is a relationship existent between the decreasing total elongation of the apical portions of the root and the lignification of desmogen cells going on at that time.

In these roots, it is observed that the inner walls of the young desmogen cells, show an immediate pitted lining of cytoplasm (figs. 3A, 4A), which with subsequent secondary wall thickening, forms the initial pitted vessel character. The fact that this pitted wall thickening is maintained can be related to the arrested elongation of surrounding cells.

The occurrence of pitted vessels in the tips of roots of seedlings grown from x-ray treated seeds, may therefore be interpreted as indicating a period of declining growth so far as it includes elongation, and as portraying the time of initiation of this decline by the extent of these pitted elements upward from the root apice. This suggests a turning point in normal elongation during the early part of germination to a decline resulting finally in a growth cessation. In the seedlings from seeds irradiated for 120 minutes, growth cessation as recognized by the failure of a further increase in length was apparent when the material was taken for histological examination, so that in them it is possible to inspect the extent of elongation during early germination and growth decline before "delayed killing" based on the types of secondary wall thickenings of the xylem elements. Other seedlings throughout the dose range of x-rays selected represent conditions further removed from "delayed killing" as the treatment was decreased. All, however, show similar indications of a decrease in extent of cellular elongation by the presence of pitted wall thickenings in the most recently formed xylem elements. This information is diagrammed in figure 8.

The manner of elongation in the roots of the treated seedlings indicated in normal elongation and in decline of elongation is also shown in the diagram. Normal elongation is recognized by the formation of the annular, spiral, scalariform and pitted vessels, as they may be, in the upper portion of the elongation region. Growth decline, climaxing with cessation of growth. based on the failure of further increase in length, is observed by the presence of pitted vessels in the lower part of the region of root elongation. The

extent of normal growth is found to decrease, and the extent of growth decline is found to increase, with increasing x-ray treatments.

The observations are briefly summarized in the following paragraphs:

1. Normal *Vicia faba* seedlings (fig. 2). Vessel formation starts with vacuolation of desmogen cells, causing the cytoplasm to form a pitted lining on the inner cell wall (fig. 2, A). The first vessel secondary wall thickening is annular, which is followed basipetally by spiral, scalariform, and pitted formations. These are considered to be related to the extent of elongation taking place in the region of the root in which they are being formed; annular and spiral in the region of rapid elongation, and scalariform and pitted in regions of slight elongation.

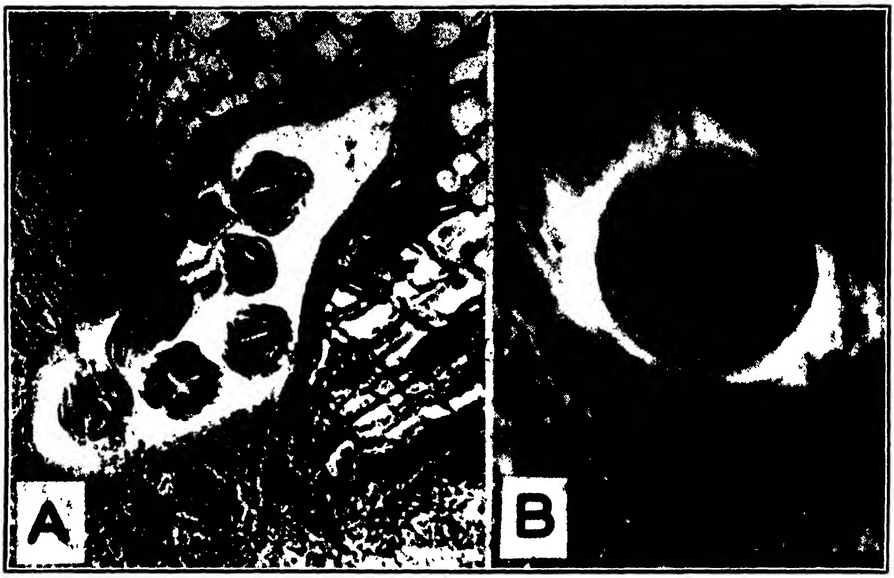

FIG. 9. Modifications about the root tip meristem in seedlings grown from seeds x-ray irradiated at 30 kv., 10 ma., for 120 minutes. A. Extensive lignification with cavities containing giant nucleus-like structures. ×450. B. Enlargement of a structure shown in A. ×about 1000.

2. Seedlings grown from seeds irradiated for 15 minutes (fig. 3). In the roots of these seedlings above the pitted vessels of the tip, a region of desmogen strands is present but is less extensive than that observed in control roots. The first vessel type is represented by a few annular elements. Spiral and scalariform thickenings are far more abundant, and are followed by a prominent region of larger pitted vessels.

3. Seedlings grown from seeds irradiated for 30 minutes (fig. 4). Above the pitted vessel present in the lowermost portion of the region of elongation, desmogen cells with vacuolated cytoplasm are evident, but these are

rapidly followed by spiral vessels with scalariform and pitted elements appearing in regions above. No annular vessels are observed.

4. Seedlings grown from seeds irradiated for 60 minutes (fig. 5). In roots of these seedlings few desmogen cells are found above the pitted vessels in the tip. Thickenings of the scalariform type appear very rapidly, and a great prominence of pitted vessels are found above. Annular and spiral elements are observed only occasionally in these roots.

5. Seedlings grown from seeds irradiated for 90 minutes (fig. 6). The region of pitted vessels is very pronounced and extends upward in these roots for approximately 6.5 mm. This is followed by a few desmogen strands which soon show wall thickenings of the scalariform type. These, however, are considerably less in number than in preceding lots. The pitted vessels appearing next are found throughout the remaining part of the region of root elongation.

6. Seedlings grown from seeds irradiated for 120 minutes (fig. 7). The vessel type in roots of these seedlings is almost completely pitted; a few scalariform elements are found approximately 8.5 mm. from the tip (fig. 8). Further inspection of these roots indicates a very extensive lignification of the tip, in which cavities are observed containing large structures which seem to represent giant nuclei. These structures are shown in figure 9, but no explanations are attempted here. The very pronounced curling of the root tip appears characteristically in seedlings irradiated with this dose of x-rays.

SUMMARY

1. Roots of *Vicia faba* seedlings grown from unsoaked seeds irradiated with soft x-rays, show a decreasing total elongation as increasing doses of x-rays are employed.

2. A histological study of such roots and of control roots affords adequate data to demonstrate experimentally that the type of wall thickening found in vessels is determined by the extent of elongation in surrounding tissues.

3. After the onset of "delayed killing" in seedlings grown from x-ray irradiated seeds, evidenced by a decline in growth as it involves elongation, only pitted vessels are formed. This is apparently related to a markedly decreased extent of elongation of cells surrounding the vessels.

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BULLETIN OF THE TORREY BOTANICAL CLUB

69(3): 235–239 MARCH, 1942

SOMATIC CHROMOSOMES OF ACONITUM NOVEBORACENSE AND A. UNCINATUM[1]

DOROTHY J. LONGACRE

The reduced chromosome number of *Aconitum napellus* was reported as 12 by Overton (7). Osterwalder (6), presumably working with the same type of material, also reported the haploid number for this species to be 12. Langlet (4) reported the chromosome number for a series of aconites in which he listed the diploid number as 16. Since the basic number has been established as 8, it is evident that Overton and Osterwalder were dealing with triploid aconites and not with diploid plants. Afify (1) reported *A. napellus* as a tetraploid having a chromosome number of 32. Schafer and La Cour (8) tabulated the chromosome number for the aconites of the blue-flowered group (*Eu-Aconitum*) as follows: diploids with 16 chromosomes, triploids with 24, tetraploids with 32, hexaploids with 48, and octoploids with 64. The diploid chromosome number of *A. noveboracense* was reported by Bonisteel (2) as 16.

The purpose of this study was to determine the chromosome number of the species of aconites which are indigenous to the Appalachian Mountains of the eastern United States. It was possible to obtain authenic material of only *A. noveboracense* (Gray) and *A. uncinatum* (L).

MATERIALS AND METHODS

Dormant tubers of *A. noveboracense* were collected along the Beaverkill in the Catskill Mountains in October 1940 and tubers of *A. uncinatum* were obtained from E. C. Robbins, Ashford, N. C. The dormant tubers of both species were potted and maintained in a resting condition until January when growth was resumed.

Root tips were fixed in Craf's modification of Nawaschin Fluid, F.A.A., and 2 Be of Schafer and La Cour. All of these fixatives proved to be satisfactory but most of the material was fixed with the Craf modification. Sections were cut from 10 to 15 microns thick and were stained by the iodine-gentian-violet technique and also by the iron-alum-hematoxylin methods. Warmke's modification of Belling's aceto-carmine smear method was used on root tips. Aceto-orcein (0.5 per cent in 45 per cent acetic acid) was also used. It was found that smears made with aceto-orcein were clearer than those made with aceto-carmine.

[1] The writer wishes to express her appreciation to Professor Wm. J. Bonisteel for materials used in this study and to Professor C. A. Berger, S.J., for constructive criticism and suggestions.

The metaphase plates and idiograms were outlined and partially drawn by the use of the camera lucida. For finer details of chromosome structure the smears made with aceto-orcein proved to be the most satisfactory. The photomicrographs were made with an oil immersion lens at a magnification of 450 diameters and subsequently enlarged to 1350 diameters.

OBSERVATION AND DISCUSSION

Examination of fixed and stained preparations and of root tip smears of *A. noveboracense* and *A. uncinatum* proved that the diploid chromosome number in somatic tissues is 16. This confirms the results of previous workers (2, 4, 8) that the basic number for these plants is 8. There is no evidence in well fixed preparations of any variation in the chromosome number of individuals of these species. It is assumed that the meiotic behavior of these plants is normal since they set seed freely in their native habitats. Seeds were also collected from the plants growing in the greenhouse.

In root tips of Sparks aconite, a triploid species, Bonisteel found the chromosome number of certain sectors to be twice the number of the normal somatic count. Schafer and La Cour also reported that the two diploid species, *A. transsectum* and a clone of *A. vulparia*, had tetraploid sectors present with diploid somatic tissue. The two species of plants under investigation were carefully examined for any sectors that would show an increase in the normal chromosome complement that might be due to a failure of spindle formation and the subsequent production of sectors which are of chromosomal chimeras. It is to be noted that the spontaneous origin of such sectors in normal somatic tissues might have a bearing upon the extended polyploid series of plants that occur in the normal range and distribution of the aconites.

The somatic chromosomes of *A. noveboracense* ($2n = 16$) are shown in figure 6 from an equatorial plate preparation at metaphase. The comparable chromosomes of *A. uncinatum* ($2n = 16$) are shown in figure 7.

The total chromosome length of *A. noveboracense* was found to be 94.8 microns and that of *A. uncinatum* to be 103 microns. The chromosome lengths recorded for the two species studied in this investigation agree with the findings of other workers. Schafer and La Cour calculated the total length of all chromosomes in the species that they studied and found that a difference exists between the chromosome length in the blue-flowered aconites (section *Eu-Aconitum*) and the yellow-flowered aconites (section *Lycoctonum*). They conclude from their studies that total chromosome length appears to give no clue to the systematic position of the aconites. The average chromosome length in the blue-flowered group listed by Schafer and La Cour was between 80.6 and 96.8 microns. *A. noveboracense* and *A. uncinatum* belong to the section *Eu-Aconitum*. The total chromosome length

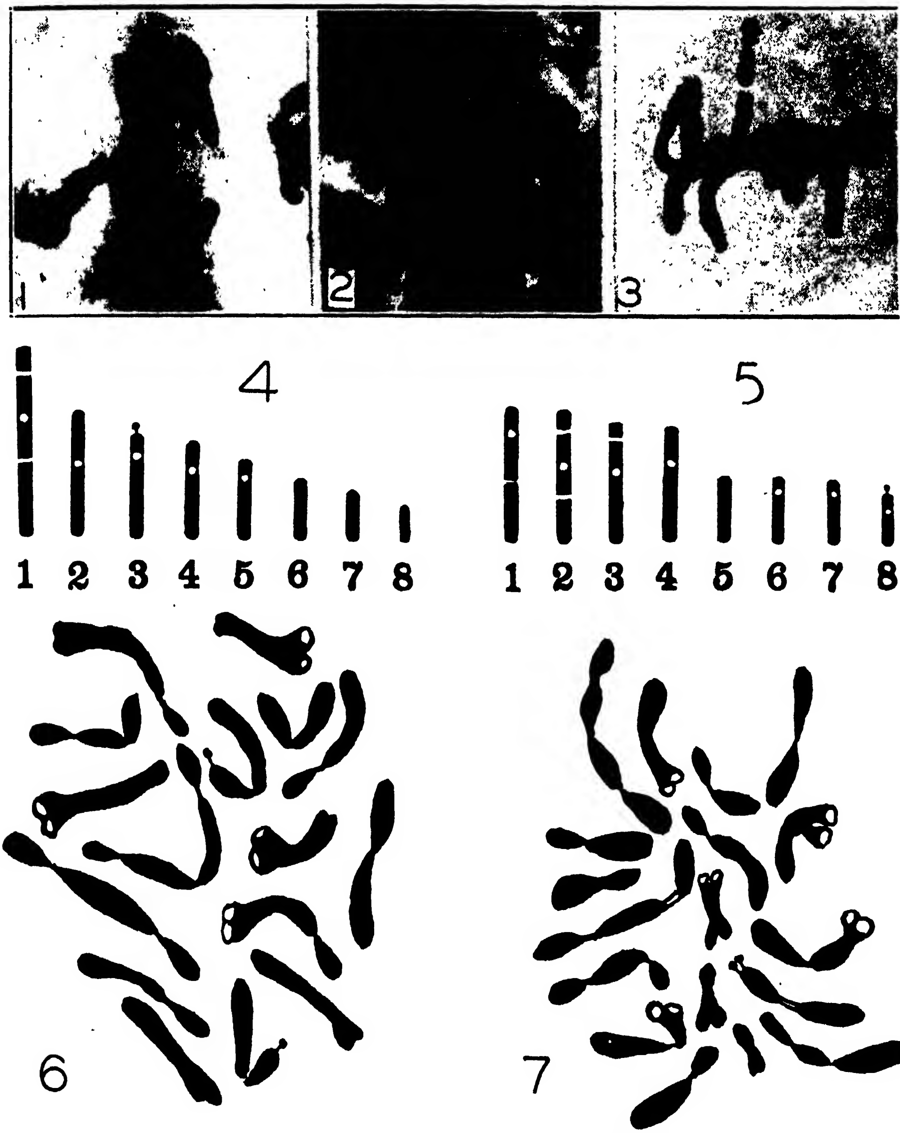

FIG. 1. *A. noveboracense*. Anaphase showing the satellites on chromosome 3. Smear method of Warmke and Belling. Aceto-orcein smear. ×1350. FIG. 2. *A. noveboracense*. Early anaphase showing one satellited chromosome and constrictions on the chromosomes that are in focus. Aceto-orcein smear method. ×1350. FIG. 3. *A. uncinatum*. Early anaphase showing constrictions at the spindle attachment region and secondary constrictions in both arms in chromosome 2. Aceto-orcein smear method. ×1350. FIG. 4. *A. noveboracense*. Idiogram with one set of chromosomes. FIG. 5. *A. uncinatum*. Idiogram with one set of chromosomes. FIG. 6. *A. noveboracense*. Metaphase plate from root tip. Fixed in Craf and stained with iodine-gentian-violet. FIG. 7. *A. uncinatum*. Metaphase plate from root tip. Fixed in Craf and stained in iron-alum-hematoxylon.

of *A. noveboracense* bears out the findings of Schafer and La Cour but that of *A. uncinatum* is somewhat greater as reported here.

When the idiogram of *A. noveboracense* (fig. 4) is compared with that of *A. uncinatum* (fig. 5) it is found that the chromosomes fall into a definite pattern in which 1, 2, 3, and 4 chromosomes from each species are characterized by their extreme length in comparison with the three or four other chromosomes of each species which are much shorter. Only one set of chromosomes is shown in figures 4 and 5. In *A. noveboracense* the chromosomes range in size from 12.9 to 1.2 microns, and in *A. uncinatum* from 9.2 to 3.2 microns. The individual chromosome lengths are given in the following table:

Chromosome designated	1	2	3	4	5	6	7	8	
A. noveboracense	12.9	7.2	6.7	6.5	5.6	4.0	3.3	1.2	microns
A. uncinatum	9.2	9.1	8.8	8.2	4.4	4.4	4.2	3.2	microns

As Longley (5) has pointed out, length measurements of chromosomes are exceedingly useful both for identification and for comparison, but they must be used with caution because of the uncertainty that chromosomes are in the same phase in different cells. Moreover, the degree of chromosome contraction varies with the kind of fixative used.

The spindle attachment region varies from terminal or subterminal to median. Darlington (3) states that *Aconitum* and *Aucuba* are exceptional plants in having long attachment constrictions in place of the short constrictions found in most plants. In chromosome 2 of *A. uncinatum* (fig. 3), as shown in smear preparations, the region of spindle attachment is extremely long. The secondary constrictions are also longer than those normally found. Darlington illustrates this type of constriction in aconite chromosome forms at mitosis.

Satellites were found in both species of aconites studied. In *A. noveboracense* one large satellite was found on chromosome 3 in figure 4 and 6. In late anaphase (fig. 1) the two satellites are shown while in figure 2 the same satellited chromosome is shown at an earlier stage. In *A. uncinatum* (fig. 5 and 7) one small satellite is illustrated on chromosome 8. Satellites were more clearly defined in smear preparations where the chromatin material was well spread.

SUMMARY

1. The somatic chromosome number of *A. noveboracense* and *A. uncinatum* is 16 ($2n = 16$).

2. Both *A. noveboracense* and *A. uncinatum* have 8 distinct types of chromosomes; four of these are long and four are short.

3. The extreme length of the secondary constrictions of *A. noveboracense* and *A. uncinatum* confirm the reports of Darlington that aconites in general are characterized by longer secondary constrictions than most plants.

4. *A. noveboracense* has one chromosome with a satellite and *A. uncinatum* has one short chromosome with a satellite.

5. The idiograms of *A. noveboracense* and *A. uncinatum* are compared as to chromosome size and shape.

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STUDIES IN THE ERICALES: A NEW NAME IN BLUEBERRIES

W. H. CAMP

It has recently been demonstrated that the low-growing, glaucous, evergreen, eglandular blueberry of the Gulf Coast is a basic, archetype diploid, and that the species (*Vaccinium myrsinites*) with which it has long been associated as a variety is an allotetraploid derived only in part from this species. While this in itself is scarcely enough to warrant recognition of both as different species, the characters which separate the two are quite as good as those traditionally accepted by more cautious students between other species of the genus. It is admitted that plants may be found, especially in western Florida, which seem to bridge the gap between *V. myrsinites* and this species. This, however, is primarily the result of a peculiar type of genic saltation within the allotetraploid *V. myrsinites* population. This phenomenon will be discussed in more detail in another place. Being preoccupied as a specific name in the genus, the name of the variety cannot be raised in rank. The following new name is therefore proposed:

Vaccinium darrowi Camp, nom. nov. *Vaccinium myrsinites* var. *glaucum* A. Gray, Synop. Fl. N. A. **2**: 21. 1878.

The species is named in honor of Dr. George M. Darrow of the United States Department of Agriculture who has been actively interested in clarifying the biological situation underlying those blueberries which are particularly promising for breeding. It is due in great part to his efforts that we have learned of the genetic situation, not only in the present species under consideration, but in other and more perplexing *Vaccinium* populations.

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69(3): 241–243 MARCH, 1942

A NEW CAMPANULA FROM IDAHO

ROGERS MCVAUGH

The mountainous areas of central Idaho are still relatively inaccessible and are, as a consequence, not well known botanically. It is therefore of peculiar interest that I am able to present evidence of the general occurrence in this region of a Campanula which appears to be endemic. The plant in question, so far as may be ascertained, is closely related to *Campanula parryi* A. Gray, which occurs in mountainous areas from southern Wyoming to northern New Mexico, west to southern Utah and northern Arizona. Although the known ranges of the two plants are not contiguous, the differences between them do not seem to be fundamental ones, and the Idaho plant may be designated as a variety of *C. parryi.*[1] Typical *Campanula parryi* may then be called var. *parryi.*[2]

CAMPANULA PARRYI var. IDAHOENSIS McVaugh

Plants perennial, glabrous except for the bases of the leaves of vegetative shoots and those of the lowermost leaves of the flowering stems (hypanthium sometimes pubescent). Rootstocks slender, much branched, the branches terminating in rosette-like clusters of leaves from which arise in summer, by elongation of the axes, the flowering stems. Flowering stems erect, 0.5–1.5 mm. in diameter at base, often with strongly ascending branches, few–35 cm. high, clustered from adjacent branches of the rootstock, usually one only arising from each branch. Leaves cauline and basal, few–15 on each stem, the lower sometimes approximate. Uppermost cauline leaves usually bract-like, linear or narrowly elliptic, 0.3–1.0 (2) mm. wide, 2–10 (25) mm. long, entire, blunt or acute. Middle cauline leaves linear to lanceolate, elliptic, or oblanceolate, mostly 2–5 mm. wide, 2–5 cm. long, acute or blunt at tip, somewhat narrowed or attenuate at base, entire or remotely and minutely callose-denticulate, the teeth usually not apparent to the unaided eye. Basal (rosette) leaves and lowermost cauline leaves elliptic, oblanceolate or occasionally obovate, rounded at tip, the base merely acute or attenuate and subpetiolate; petiole-like bases (at least of the rosette leaves) with stiff white cilia on the margins. Blades of these leaves 3–12 mm. wide, 7–28 mm. long, including petiolar base, usually 2–3 (5) times as long as wide, often remotely denticulate but the teeth usually not apparent to the unaided eye. Margins

[1] *Campanula parryi* A. Gray, var. **idahoensis** McVaugh, var. nov., a planta typica foliis integris, lobis calycis maturi 2–6 (raro 9) mm. longis integris, pedunculis terminalibus 0.2–1.5 (raro 5) cm. longis, corollis 9–15 mm. longis differt. Type: "Swamp Lake, on trail from Kooskooska Meadows to Friday's Pass, Selway Forest, Idaho, 7000 ft.," *J. E. Kirkwood & J. W. Severy 1638*, August 26, 1923; in the Herbarium of Montana State University, Missoula.

[2] *Campanula parryi* A. Gray, var. **parryi** McVaugh, nom. nov. *Campanula parryi* A. Gray, Syn. Fl. ed. 2, 2¹: suppl. 395. 1886.

of all leaves usually thickened and minutely papillose-roughened. Flowers erect or slightly nodding, terminating the stems, the uppermost leaf (bract) 2–15 (rarely 50) mm. below the flower which terminates the main axis. Additional flowers, if present, 1–3, terminating the axillary branches. Hypanthium in anthesis cup-shaped, campanulate or obconic, glabrous, or sparsely or densely beset with short white flaccid hairs, 2–4 mm. long, usually slightly longer than broad when pressed, rounded or attenuate at base but usually with an abrupt break between hypanthium and peduncle. Capsule erect, campanulate, turbinate or ellipsoid, broadest at or above the middle, about 3 mm. in diameter by 7–11 mm. long, wholly inferior, attenuate or abruptly rounded at base, opening near the summit by three upcurled valves. Seeds ellipsoid-lenticular, about 1 mm. long, brown, very minutely lined. Calyx-lobes narrowly triangular or subulate, acute, entire, 1–2.5 mm. wide at base, 2–6 mm. long in anthesis, usually elongating in fruit, becoming (3) 6–9 mm. long; lobes appressed or somewhat spreading in flower and fruit. Corolla glabrous, broadly campanulate, 9–15 mm. long, the width when pressed usually equalling the length or slightly exceeding it. Lobes 3–6.5 mm. long, one-third to one-half the length of the whole corolla, ovate-deltoid, acute or apiculate, the sinuses V-shaped. Style 7–13 mm. long, straight, divided 1.5–2.5 mm., roughened distally more than half its length (5–8 mm.). Anthers glabrous, linear, straight, 4–5.5 mm. long. Filaments 1.5–2 mm. long, the expanded basal part rounded, ciliate, 1–1.5 mm. long, the linear distal part hyaline, about 0.5 mm. long.

Most of the species of the genus *Campanula* are notable for extreme variation in size of corolla, in number of flowers per plant, and in habit. Definitive characters pertaining to species differentiation often reside in the fruit only. For these reasons most herbaria contain numerous wrongly determined specimens of *Campanula* which have been identified while in flower only, or identified by means of keys based upon vegetative characters or those of the flower alone. It is thus thought desirable to present at this point a key for the separation of the species with which *Campanula parryi* may be confused. In the Rocky Mountain region, at high altitudes, there occur three species, all of which occasionally bear but a single flower; *C. uniflora* regularly produces but one flower on each stem, but it is clearly unsafe to refer a plant to this species by this character alone, as *C. parryi* and *C. rotundifolia*, especially under adverse conditions of habitat, may do the same thing.

KEY TO THE SPECIES OF CAMPANULA OF THE ROCKY MOUNTAIN REGION, SOUTH OF THE CANADIAN BORDER

1. Anthers 1.5–2.5 mm. long; flower regularly 1 only, erect; capsule erect, opening by valves near the summit .... *C. uniflora* L.
1. Anthers 4–6.5 mm. long 2
   2. Bases of the rosette leaves and lower cauline leaves ciliate with white hairs up to 0.7 mm. long, the plants otherwise glabrous; flower regularly 1 only (or with 1–4 additional flowers on subordinate lateral branches); flower and capsule erect, the latter opening by valves near the summit .... (*C. parryi*) 3

3. Leaves and calyx-lobes entire, the teeth, if any, scarcely visible to the unaided eye; calyx-lobes 2–6 (9) mm. long in fruit; corolla 9–15 mm. long, the principal peduncle 0.2–1.5 (rarely 5) cm. long .... var. *idahoensis*

3. Calyx-lobes and at least some of the leaves usually with teeth plainly visible to the unaided eye; calyx-lobes (6) 10–15 mm. long in fruit; corolla (9) 15–23 mm. long, the principal peduncle usually 3–5 cm. long .... var. *parryi*

2. Plants glabrous or hispidulous, the leaf-bases never long-ciliate but at most with hispid margins, the processes up to 0.2 mm. long; flowers regularly numerous, sometimes one only; capsule nodding, opening by valves at base; flowers erect or nodding .... *C. rotundifolia* L.

It should be noted that I am including under *Campanula rotundifolia* all the plants which have passed as *C. petiolata* A. DC., as well as *C. macdougalii* Rydb. and *C. sacajaweana* M. E. Peck. I have examined the type specimens of the two latter, and in my judgment neither is sufficiently distinct to be set up as a distinct species.

I have seen the following material of *C. parryi* var. *idahoensis:*

IDAHO—ADAMS CO.: Pyramid Peak, Weiser N. F., *Christ 8684,* Aug. 8, 1937 (herb. of J. H. Christ). IDAHO CO.: Hoodoo Lake, 18 mi. s.e. of Powell R. S., *Christ 12807* July 21, 1941 (U. S. National Arboretum); dry mountain top near Powell R. S., R14E, T 35N, *R. J. Davis 3629,* June 29, 1941 (herb. Univ. of Idaho, Pocatello); Buffalo Hump, near Orogrande, in tundra, *Christ 11606,* July 31, 1940 (herb. Christ); Monroe Creek, trail crossing in Clearwater Forest, *Kirkwood 2002,* Aug. 26, 1924 (herb. Mont. State); near Friday's Pass, 7000 ft., *Kirkwood & Severy 1649,* Aug. 26, 1923 (herb. Mont. State); Friday's Pass, 7940 ft., *Kirkwood & Severy 1639* (herb. Mont. State); trail from Kooskooska Meadows to Friday's Pass, Swamp Lake, *Kirkwood & Severy 1637* and *1638* (Type) (both in herb. Mont. State).

MONTANA—MISSOULA CO.: Lolo Road, sunny site, hillside, *F. H. Rose 538,* July 19, 1938 (U. S. National Arboretum).

69(3): 244–248 MARCH, 1942

NEW COMBINATIONS AND NEW NAMES IN THE UMBELLIFERAE—II[1]

MILDRED E. MATHIAS AND LINCOLN CONSTANCE

LOMATIUM Raf. Journ. Phys. **89**: 101. 1819. *Cogswellia* Spreng. in Linn. Syst. Veg. ed. Roem. & Schult. **6**: 48. 1820. *Euryptera* Nutt. ex Torr. & Gray, Fl. N. Am. **1**: 629. 1840. *Leptotaenia* Nutt. ex. Torr. & Gray, op. cit. p. 629. *Leibergia* Coult. & Rose, Contr. U. S. Nat. Herb. **3**: 575. 1896. *Cynomarathrum* Nutt. ex Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 244. 1900. *Cusickia* Jones, Contr. West. Bot. **12**: 39. 1908. *Peucedanum* and *Ferula* of American authors, not of Linn. 1753.

The genera *Lomatium* and *Leptotaenia* have been kept distinct by all authors treating the American Umbelliferae. The diagnostic differences were summarized by Coulter and Rose[2] as follows: "*Peucedanum* [to which the species of *Lomatium* were then referred] also differs in its membranous lateral wings, which are strongly nerved on the ventral face at the inner margin, in the absence of a longitudinal ridge on the commissural surface, and in the often solitary oil-tubes. In the case of those species of *Peucedanum* which have more than one oil-duct in the intervals, the decidedly membranous wings are in sharp contrast with those of *Leptotaenia*. . . ." Subsequent writers have combined the nature of the fruit wings with features of habit and size to distinguish the two assemblages of species.

The longitudinal ridge on the commissural surface of the mericarps is to be found in many if not all of the species of *Lomatium* as well as in those of *Leptotaenia*, and the wing-nervation appears to have little reliability. Solitary oil tubes are characteristic of *Leptotaenia Leibergi* Coult. & Rose, *L. minor* Rose and *L. salmoniflora* Coult. & Rose. As to habit, the species of *Leptotaenia* are usually stated to be tall, stout and caulescent, with broad leaves, whereas the species of *Lomatium* are described as less stout, low and acaulescent or nearly so, with smaller leaves. It is true that *Leptotaenia dissecta* Nutt. and its var. *multifida* (Nutt.) Jepson, *L. purpurea* (Wats.) Coult. & Rose and *L. californica* Nutt. are all tall, stout and caulescent with very broad leaves. However, *Lomatium Suksdorfii* (Wats.) Coult. & Rose and its var. *Thompsonii* Mathias and *L. triternatum* (Pursh) Raf. vars. *anomalum* (Jones) Mathias and *macrocarpum* (Coult. & Rose) Mathias are equally large, robust and caulescent, with leaves of comparable size. *Leptotaenia anomala* Coult. & Rose, *L. Bradshawii* Rose, *L. Leibergi, L. minor, L. salmoniflora* and *L. Watsonii* Coult.

[1] The first paper of this series was published in Bull. Torrey Club 68: 121–124. 1941.
[2] Rev. N. Am. Umbel. 51. 1888.

& Rose, on the other hand, are all relatively small plants, which are either acaulescent or short-caulescent, and provided with proportionately small leaves.

The habital similarity between certain species customarily assigned to *Leptotaenia* and others retained in *Lomatium* is both striking and confusing. Thus, *Leptotaenia anomala* is scarcely distinguishable in flower from *Lomatium caruifolium* (Hook. & Arn.) Coult. & Rose and *Lomatium marginatum* (Benth.) Coult. & Rose, and all are closely related by their several, obscure oil tubes and semi-orbicular or obovate fruit. *Leptotaenia Bradshawii* is habitally very similar to *Lomatium leptocarpum* (Nutt.) Coult. & Rose, and grows in nearly identical habitats—moist swales. *Leptotaenia salmoniflora* is scarcely separable from *Lomatium Grayi* Coult. & Rose unless either fresh flowers or ripe fruit are present. *Leptotaenia Leibergi* so closely resembles such bulbous species of *Lomatium* as *L. Canbyi* Coult. & Rose that it had been previously described as *Peucedanum* (*Lomatium*) *Hendersoni* Coult. & Rose. It is clear from the above that differences in habit may permit the separation of certain species of the two genera from each other, but does not in itself render the two groups of species generically distinct.

We must necessarily turn, then, to a re-examination of the characters of the fruit, which have furnished the chief technical grounds for retaining the two genera. The typical *Leptotaenia* fruit, that found in *Leptotaenia dissecta* and var. *multifida*, *L. purpurea* and in most plants of *L. californica*, superficially resembles a pumpkin seed: the wings are very thick and corky, and form a prominent, narrow border to the mericarp. In *L. Leibergi*, *L. minor*, *L. salmoniflora* and *L. Watsoni* the dorsal surface of the mericarp and of the wing are uniformly brown in color, with the result that the only slightly thickened wing is scarcely visible unless the ventral surface or a transverse section of the mericarp is examined. The fruit of *L. anomala* is very much like that of *Lomatium caruifolium*, which has "thickish" wings, and *L. marginatum*, except that it is somewhat more corky-thickened. *Leptotaenia californica* is remarkable for the fact that fruit from the southern portion of the range has the "pumpkin seed" form, while that from the northern limits is similar, but thin-winged. The traditional generic distinction thus breaks down in this one species.

It seems evident that the single technical distinction of wing thickness has been over-emphasized to keep apart under *Leptotaenia* an unnatural group of species, the members of which show close relationships to different species of *Lomatium*. Accordingly, we find it necessary to incorporate *Leptotaenia* in the genus *Lomatium*. This change requires the following new combinations and new names, the first of which has already been indicated in our herbarium by Dr. Robert F. Hoover.

Lomatium humile (Coult. & Rose) Hoover, comb. nov. *Leptotaenia anomala* Coult. & Rose, Rev. N. Am. Umbel. 53. 1888, not *Lomatium anomalum* Jones, 1900. *Leptotaenia humilis* Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 200. 1900. *Leptotaenia humilis* var. *denticulata* Jepson, Madrono **1**: 146. 1923. *Lomatium caruifolium* var. *denticulatum* Jepson, op. cit., p. 151. 1924.

Lomatium Bradshawii (Rose ex Mathias) Mathias & Constance, comb. nov. *Leptotaenia Bradshawii* Rose ex Mathias, Lflts. West. Bot. **1**: 101. 1934.

Lomatium dissectum (Nutt.) Mathias & Constance, comb. nov. *Leptotaenia dissecta* Nutt. ex Torr. & Gray, Fl. N. Am. **1**: 630. 1840. *Leptotaenia dissecta* var. *foliosa* Hook. Lond. Journ. Bot. **6**: 236. 1847. *Cynapium Bigelovii* Torr. Pacif. RR. Rept. **4**: 94. 1856. *Ferula dissecta* Gray, Proc. Am. Acad. **7**: 348. 1868. *Ferula dissoluta* Wats. in Brewer & Wats. Bot. Calif. **1**: 271. 1876. *Leptotaenia foliosa* Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 198. 1900.

LOMATIUM DISSECTUM (Nutt) Mathias & Constance var. **multifidum** (Nutt.) Mathias & Constance, comb. nov. *Leptotaenia multifida* Nutt. ex Torr. & Gray, Fl. N. Am. **1**: 630. 1840. *Ferula multifida* Gray, Proc. Am. Acad. **7**: 348. 1868. *Leptotaenia Eatoni* Coult. & Rose, Rev. N. Am. Umbel. 52. 1888. *Leptotaenia multifida* var. *Eatoni* Jones, Contr. West. Bot. **12**: 40. 1908. *Leptotaenia dissecta* var. *multifida* Jepson, Madrono **1**: 145. 1923.

Lomatium columbianum Mathias & Constance, nom. nov. *Ferula purpurea* Wats. Proc. Am. Acad. **21**: 453. 1886, not *Lomatium purpureum* A. Nels. 1901. *Leptotaenia purpurea* Coult. & Rose, Rev. N. Am. Umbel. 52. 1888.

Lomatium salmoniflorum (Coult. & Rose) Mathias & Constance, comb. nov. *Leptotaenia salmoniflora* Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 201. 1900.

Lomatium minus (Rose) Mathias & Constance, comb. nov. *Leptotaenia minor* Rose ex Howell, Fl. N. W. Am. **1**: 251. 1898. *Cusickia minor* Jones, Contr. West Bot. **12**: 40. 1908.

Lomatium cuspidatum Mathias & Constance, nom. nov. *Leptotaenia Watsoni* Coult. & Rose, Rev. N. Am. Umbel. 52. 1888, not *Peucedanum Watsoni* Coult. & Rose, 1888, nor *Lomatium Watsoni* Coult. & Rose, 1900.

Lomatium californicum (Nutt.) Mathias & Constance, comb. nov. *Leptotaenia californica* Nutt. ex Torr. & Gray, Fl. N. Am. **1**: 630. 1840, not *Peucedanum californicum* Nutt., 1840, nor Coult. & Rose, 1888. *Ferula californica* Gray, Proc. Am. Acad. **7**: 348. 1868. *Leptotaenia californica* var. *platycarpa* Jepson, Erythea, **1**: 8. 1893. *Leptotaenia californica* var. *dilatata* Jepson, op. cit., p. 63.

PODISTERA Wats. Proc. Am. Acad. **22**: 475. 1887. *Ligusticella* Coult. & Rose, Contr. U. S. Nat. Herb. **12**: 445. 1909. *Orumbella* Coult. & Rose, op. cit., p. 446.

In a recent paper,[3] the authors pointed out the untenability of the genus *Orumbella* and transferred its sole species to *Ligusticella,* hitherto also represented by a single species. It was surprising, therefore, to be unable to find valid generic characters to separate *Ligusticella,* thus reconstituted, from *Podistera,* a third reputedly monotypic genus. *Podistera* is based upon *P. nevadensis* (Gray) Wats., a species first questionably referred to *Cymopterus* Raf. by Gray, who had at hand only inadequate material. The senior author has already suggested[4] that *Podistera* differs markedly from *Cymopterus* in the possession of a stylopodium and in the lack of wing-development of the fruit. The compression of the fruit, moreover, is lateral in *Podistera* and dorsal in *Cymopterus,* which indicates that these genera are scarcely consanguine. On the other hand, *Podistera* agrees with *Ligusticella* in these and all other important diagnostic features. We are thus forced to combine the three supposedly monotypic genera into a single genus. The resultant genus has a remarkable distribution, with one species in Alaska, a second alpine in California, and the third alpine in Colorado. The following new combinations are needed:

Podistera Eastwoodae (Coult. & Rose) Mathias & Constance, comb. nov. *Ligusticum Eastwoodae* Rose ex Eastw. Zoe **4**: 17. 1893, nomen nudum; Coult. & Rose, Contr. U. S. Nat. Herb. **3**: 320. *pl. 13*. 1895. *Ligusticella Eastwoodae* Coult. & Rose, Contr. U. S. Nat. Herb. **12**: 445. 1909.

Podistera Macounii (Coult. & Rose) Mathias & Constance, comb. nov. *Ligusticum Macounii* Coult. & Rose, Contr. U. S. Nat. Herb. **1**: 289. *pl. 23*. 1893. *Orumbella Macounii* Coult. & Rose, Contr. U. S. Nat. Herb. **12**: 446. 1909. *Ligusticella Macounii* Mathias & Constance, Bull. Torrey Club **68**: 123. 1941.

PRIONOSCIADIUM Wats. Proc. Am. Acad. **23**: 275. 1888. *Langlassea* Wolff ex Fedde, Repert. **9**: 420. 1911.

PRIONOSCIADIUM THAPSOIDES (DC.) Mathias var. **Pringlei** (Wats.) Mathias & Constance, comb. nov. *Prionosciadium Pringlei* Wats. Proc. Am. Acad. **23**: 276. 1888.

PTERYXIA Nutt. ex Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 170. 1900. *Cymopterus* § *Pteryxia* Nutt. ex Torr. & Gray, Fl. N. Am. **1**: 624. 1840. *Pseudopteryxia* Rydb. Bull. Torrey Club **40**: 71. 1913. *Pseudoreoxis* Rydb. op. cit., p. 73.

Three species, which have heretofore collectively found a place in *Pseudocymopterus* Coult. & Rose and have individually been referred also to *Cymopterus, Aletes* Coult. & Rose and *Pseudopteryxia,* have not exhibited complete conformity with any of these groups. The habit, the prominent rather than obsolete to evident calyx lobes and the membranous rather than spongy wings of the fruit relate these species more closely to

[3] Bull. Torrey Club **68**: 123. 1941.

[4] Ann. Mo. Bot. Gard. **17**: 221, 245, 248. 1930.

Pteryxia than to the type species of *Pseudocymopterus*. We believe that referring these species to *Pteryxia* will result in a more natural classification. The following transfers are required:

Pteryxia anisata (Gray) Mathias & Constance, comb. nov. *Cymopterus ? anisatus* Gray, Proc. Acad. Phila. **1863**: 63. 1864. *Pseudocymopterus anisatus* Coult. & Rose, Rev. N. Am. Umbel. 75. 1888. *Pseudocymopterus aletifolius* Rydb. Bull. Torrey Club **31**: 574. 1904. *Pseudopteryxia anisata* Rydb. Bull. Torrey Club **40**: 71. 1913. *Pseudopteryxia aletifolia* Rydb. op. cit., p. 72.

Pteryxia Hendersoni (Coult. & Rose) Mathias & Constance, comb. nov. *Pseudocymopterus Hendersoni* Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 190. 1900. *Pseudopteryxia longiloba* Rydb. Bull. Torrey Club **40**: 72. 1913. *Pseudopteryxia Hendersoni* Rydb. Fl. Rocky Mts. 624, 1064. 1917; ed. 2. 624. 1922. *Pseudocymopterus anisatus* var. *longilobus* Tidestrom, Contr. U. S. Nat. Herb. **25**: 399. 1925. (Fl. Utah & Nevada).

Pteryxia Davidsoni (Coult. & Rose) Mathias & Constance, comb. nov. *Aletes ? Davidsoni* Coult. & Rose, Contr. U. S. Nat. Herb. **7**: 107. 1900. *Pseudocymopterus filicinus* Wooton & Standl. Contr. U. S. Nat. Herb. **16**: 158. 1913. *Pseudocymopterus Davidsoni* Mathias, Ann. Mo. Bot. Gard. **17**: 282, 316. 1930.

DEPARTMENT OF BOTANY, UNIVERSITY OF CALIFORNIA
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INDEX TO AMERICAN BOTANICAL LITERATURE

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Papers that relate exclusively to bacteriology, forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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PLANT TAXONOMY AND FLORISTICS
(exclusive of fungi)

(See also under Morphology: Williams; Withner. Under Genetics: Baldwin & Culp; Hubricht & Anderson)

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MORPHOLOGY

(including anatomy, and cytology in part)

(See also under Taxonomy: Fasolo; Hollingshead. Under Mycology: Johnson & Brown. Under Genetics: Coleman)

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BULLETIN OF THE TORREY BOTANICAL CLUB
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STUDIES IN ANTIBIOSIS BETWEEN BACTERIA AND FUNGI—III. INHIBITORY ACTION OF SOME ACTINOMYCETES ON VARIOUS SPECIES OF FUNGI IN CULTURE[1]

CONST. J. ALEXOPOULOS AND J. ARTHUR HERRICK

In a previous article (1) it was reported that of 80 *Actinomyces* species grown with *Colletotrichum gloeosporioides* on agar, 14 proved to be strong inhibitors, 31 weak inhibitors, and 35 had no visible effect upon the growth of that fungus and consequently were classified as non-inhibitors.

In order to determine whether inhibition is a function of the *Actinomyces* alone or of both the *Actinomyces* and the fungus, two species of *Actinomyces* from each of the above categories were selected and grown with each of nine species of fungi representing various fungous groups.

MATERIALS AND METHODS

The same technique used in previous experiments (1), was adopted again, of inoculating the *Actinomyces* in two places on the agar, 4 cm. apart, and five days later inoculating the fungus midway between the resulting *Actinomyces* colonies. As before, the criterion of inhibition was either a flattening of the fungous colonies on the sides nearest the *Actinomyces* colonies, or a distinct general stunting of the fungous colony as compared with the growth of the control plates in which the fungus was grown alone (1, 2).

The organisms used in these studies were as follows:

Actinomycetes:
1. Strong inhibitors of *C. gloeosporioides* (1).
   - *Actinomyces annulatus* Beij.
   - *Actinomyces madurae* (Vinc.) Lehm. et Neum.
2. Weak inhibitors of *C. gloeosporioides* (1).
   - *Actinomyces flavovirens* Waks.
   - *Actinomyces gougeroti* Duché
3. Non-inhibitors of *C. gloeosporioides* (1).
   - *Actinomyces hasltedii* Waks. et Curt.
   - *Actinomyces viridochromogenus* Kr.

Fungi:
- *Phycomyces blakesleeanus* Burg.
- *Acanthorhynchus vaccinii* Shear
- *Sclerotinia sclerotiorum* (Lib.) Massee
- *Glomerella cingulata* (Stonem.) Sp. et v. Schr.
- *Phoma betae* Frank

[1] A preliminary report of this investigation was presented at the Cleveland meeting of the Ohio Academy of Science, May 9, 1941.

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Botrytis cinerea Pers.
Alternaria solani (Ell. et Mart.) J. et Gr.
Stereum gausapatum Fries, Strain S–12 (3)
Fomes fraxinophilus (Pk.) Sacc.

The medium used was prepared according to the following formula:

Glucose	30 gms.
Peptone	5 gms.
K_2HPO_4	1 gm.
Agar	20 gms.
Water	1000 cc.

The pH of the agar was adjusted to 8.1 and after sterilization at 15 lbs. for 15 minutes it was found to be 6.8. Fifteen cubic centimeters of agar were

TABLE 1. *Growth of nine species of fungi on glucose agar, grown in association with six species of Actinomyces, expressed in terms of average diameters, in millimeters, of at least four cultures*

Fungi	Age of fungous colonies in days	Control	*A. halstedii*	*A. viridochromogenus*	*A. flavovirens*	*A. gougeroti*	*A. annulatus*	*A. madurae*
			Actinomycetes					
Phycomyces blakesleeanus	3	73 × 76	64 × 68	68 × 72	62 × 70	5 × 11	46 × 62	41 × 66
	5	90 × 90[a]			.	8 × 39		.
	10	.....	.....			8 × 90	.....	.....
Acanthorhynchus vaccinii	3	11 × 13	15 × 16	15 × 16	16 × 16	5 × 5	17 × 18	15 × 17
	5	16 × 20	25 × 30	30 × 31	28 × 30	10 × 13	26 × 32	23 × 32
	10	32 × 32	. ..			16 × 35		.
Sclerotinia sclerotiorum	3	25 × 27	35 × 45	45 × 40	45 × 45	11 × 30	25 × 39	17 × 45
	5	90 × 90	.....			20 × 90	34 × 90	.
	10	...	.....		.....	26 × 90	. .	....
Glomerella cingulata	3	38 × 38	32 × 40	32 × 36	34 × 36	14 × 21	29 × 35	19 × 38
	5	72 × 73	.....	.	.	17 × 40	38 × 68	29 × 73
	10	. .	. ..		. . .	24 × 83	. .	.....
Phoma betae	3	30 × 30	31 × 32	29 × 29	28 × 29	9 × 12	27 × 31	20 × 30
	5	59 × 59	.....	34 × 58	34 × 58	11 × 20	35 × 58	29 × 58
	10	...	.....		36 × 90	15 × 50	.	. .
Botrytis cinerea	3	28 × 27	27 × 30	29 × 29	29 × 33	7 × 8	24 × 29	16 × 29
	5	70 × 70	.....	.	33 × 76	17 × 24	33 × 75	24 × 73
	10	.....	.....		..	28 × 90	.	36 × 90
Alternaria solani	3	33 × 32	31 × 32	32 × 35	31 × 32	8 × 16	25 × 32	23 × 33
	5	57 × 58	.....	..	36 × 58	16 × 30	36 × 58	30 × 58
	10	.....	.....			20 × 72	....	.....
Stereum gausapatum	3	14 × 15	15 × 16	21 × 20	18 × 18	8 × 10	16 × 16	14 × 14
	5	33 × 33	27 × 30	43 × 49	30 × 43	13 × 19	35 × 35	31 × 38
	10	70 × 70	.....		38 × 90	21 × 57	.....	.....
Fomes fraxinophilus	3	9 × 9	10 × 10	10 × 10	10 × 10	trace	10 × 10	10 × 10
	5	18 × 18	16 × 19	21 × 21	18 × 18	7 × 8	20 × 20	17 × 20
	10	42 × 42	35 × 40		33 × 37	16 × 16	37 × 44	30 × 42

[a] 90 × 90 denotes that the whole surface of the agar is covered with fungous growth.

poured into each Petri dish. Six plates were inoculated for each test as well as six controls for each fungus. Each figure presented is based on the average of at least four plates. Two measurements of the diameter of each fungous colony were taken at intervals of 24 hours beginning 2 days after the inoculation of the fungus and continuing for 8 days, or until the fungous and *Actinomyces* colonies were in contact.

RESULTS

The measurements obtained as outlined above are summarized in table 1. The first figure represents the average diameter of the fungous colony in the direction of the *Actinomyces* colonies. This figure itself, in table 1, sometimes exceeds 40 mm., which is the distance between the *Actinomyces* colonies, because the fungous sometimes grows over the *Actinomyces* or completely surrounds it, in the interval between 24-hour measurements. This may happen even after temporary inhibition of fungous growth by a weak inhibitor has taken place. The second figure represents the average diameter of the fungous colony at right angles to the first. At the first signs of inhibition, the distance between the fungus and *Actinomyces* was measured on both sides and the average recorded as "inhibitory distance" (2). Inhibitory distances between various combinations of fungi and actinomycetes are summarized in table 2.

TABLE 2. *Average inhibitory distances between 6 species of Actinomyces and 9 species of fungi, expressed in millimeters*

Fungi	*A. halstedii*	*A. viridochromogenus*	*A. annulatus*	*A. madurae*	*A. flavovirens*	*A. gougeroti*
Phycomyces blakesleeanus	0	0	6	5	3	15
Acanthorhynchus vaccinii	7	0	8	9	6	15
Sclerotinia sclerotiorum	1	0	7	12	1	15
Glomerella cingulata	1	3	9	11	1	13
Phoma betae	1–	1	5	12	2	14
Botrytis cinerea	5	0	7	13	3	16
Alternaria solani	0	1 –	5	11	1–	13
Stereum gausapatum	5	1–	0	3	3	13
Fomes fraxinophilus	9	2	3	10	3	15

DISCUSSION

Employing the same standards as in previous experiments (1), i.e., classifying actinomycetes as "strong inhibitors" if the inhibitory distance at which they become effective is 10 or more millimeters, and as "weak in-

hibitors'' if the inhibitory distance is less than 10 mm., we may now reclassify the six species used in these experiments with respect to their effect upon the growth of the nine fungi against which they were tested.

The four actinomycetes selected from those which inhibited *C. gloeosporioides* in previous experiments (1), also inhibit the nine fungi employed here with the exception that *A. annulatus* fails to inhibit *S. gausapatum*.

A. halstedii and *A. viridochromogenus,* which had been classified as non-inhibitors of *C. gloeosporioides* (1), may or may not inhibit other fungi, but when they do inhibit, they do so weakly, as shown by their inhibitory distances. An exception is found with *A. halstedii* against *F. fraxinophilus,* between which the inhibitory distance is rather great; this is shown in table 2.

There was considerable discrepancy in the behavior of strong and weak inhibitors against various fungi. *A. annulatus*, which was selected as a strong inhibitor, proved to be a weak inhibitor in all tests but one in which it failed to inhibit the growth of the fungus. On the other hand, *A. madurae*, also selected as a strong inhibitor, proved to be a strong inhibitor in six tests and a weak inhibitor in three (table 2). *A. flavovirens* and *A. gougeroti* were selected as weak inhibitors on the basis of their effect on *C. gloeosporioides* (1). *A flavovirens* was a weak inhibitor of all other fungi as well, but *A. gougeroti* proved to be a very strong inhibitor of all other fungi employed in these experiments (table 2).

In evaluating the results discussed above, it must be borne in mind that no natural dividing line exists between so-called strong and weak inhibitors, and that the inhibitory distance of 10 mm. which is used as a dividing line between the two categories has been selected arbitrarily. As shown in table 2 the inhibitory distances vary from less than 1 to 16 mm.

It will be noted from the figures presented in table 1, that, in some cases, the fungi, notably *Acanthorhynchus vaccinii* seem to grow better in the presence of some actinomycetes than in the control plates; in spite of that, inhibitory distances between colonies of these organisms are recorded in table 2. A further examination of the figures in such cases will show that the diameter of the fungous colony between the Actinomyces colonies (first figure) is considerably smaller than the diameter at right angles (second figure), showing that as the margin of the fungous colony approached the *Actinomyces* its rate of growth was retarded as compared to the rate of growth at right angles. Furthermore, such fungous colonies were generally distinctly flattened on the sides nearest the *Actinomyces* colonies leaving no doubt as to the inhibitory action taking place. Photographs of this effect have been published in a previous article (2).

On the other hand, the fact that in some cases the fungus grows better,

in at least one direction, in the presence of the *Actinomyces* than in the controls, may very well be an indication of the presence of growth stimulating substances secreted in the agar in addition to, or instead of, the inhibitory substances. That such growth stimulating substances are manufactured by various microorganisms is well established. This phase of the problem is being investigated.

SUMMARY AND CONCLUSIONS

1. Actinomycetes differ in their ability to inhibit the growth of a given fungus in culture under the conditions of the experiment.

2. Fungi differ in their susceptibility to inhibition by a given species of *Actinomyces*.

3. The degree of inhibition of fungous growth is a function of both the fungus and the inhibiting organism.

4. No correlation seems to exist between the normal rate of growth of a fungus and the degree of its inhibition by a given *Actinomyces*.

5. No correlation seems to exist between the relative position of a fungus in the accepted system of classification and the degree of its inhibition by a given *Actinomyces*.

6. There is some evidence that some of the species of *Actinomyces* employed in these experiments, may be manufacturing a growth promoting substance in addition to, or instead of growth inhibiting substances.

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A POLLEN STUDY OF LAKE SEDIMENTS IN THE LOWER WILLAMETTE VALLEY OF WESTERN OREGON[1]

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The occurrence of peat deposits beyond the limits of Pleistocene glaciation in the Pacific Northwest is uncommon. Sphagnum bogs are rare except along the Pacific Ocean where the moist, equable climate is favorable for their development. In the Willamette Valley of western Oregon, the small amount of precipitation during the growing season is probably the chief reason for the absence of sphagnum bogs. The author has made a pollen study of the only extensive one known to exist in this area (Hansen 1941a). Sedge, tule, reed, and cat-tail swamps or bogs are also uncommon, but they may be occasionally found on floodplains, in abandoned stream channels, and in oxbow lakes. Many of these areas have been drained for agricultural purposes, and often practically all evidence of their previous existence has been obliterated. Floodplain associes of willow, cottonwood, alder, ash, and large-leaf maple also mark the presence of hydroseres that are rapidly nearing the climax stage of plant succession. Many of these areas are merely alluvial floodplains where the water table remains sufficiently high during the summer to permit the growth of moisture-loving species. Few of these areas, however, have an accumulation of peat or other pollen-bearing sediments suitable for the purpose of pollen analysis. It is fortunate, therefore, that several peat deposits satisfactory for pollen analysis are present in the lower Willamette Valley. Their pollen record serves as a valuable accession to the problem of post-Pleistocene forest succession and climate in the Pacific Northwest. The Willamette Valley is a rather distinct floristic province within an otherwise more or less homogeneous hemlock-cedar forest climax of this region.

ORIGIN AND CHRONOLOGY OF THE SEDIMENTS

The peat profiles for this study were obtained from two different deposits. Two profiles were obtained from the Labish Flats, located about 4 miles northeast of Salem, Oregon. Lake Labish was formerly a shallow lake before it was drained in 1912 for agricultural purposes. The lacustrine sediments cover an area about 9 miles long and one-half mile wide. The elevation is between 140 and 150 feet above sea level. The upper end of the former lake is separated from the Willamette River by a divide not over two miles wide and about 30 feet high. The width and depth of the channel and the adjacent

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topography indicate that it is an abandoned course of the Willamette River. The channel was probably occupied by a distributary of a braided stream. The lake was evidently ponded by a delta deposited by the Pudding River. Later the Willamette River receded and the present channel carried its water. The Pudding River follows the old Willamette Channel from the lower end of Lake Labish, emptying into the present Willamette River farther north and downstream. The level of Lake Labish was maintained by smaller streams debouching into it. One of them is now the Little Pudding River.[2] Peat samples of the profiles were taken at quarter-meter intervals with a Hiller peat borer. One profile was taken in section 31 of T. 6 S., R. 2 W., and the other was obtained in section 21 of the same township. The depth of the lacustrine sediments in both profiles is 7 meters. The depth has been somewhat reduced since drainage, by consolidation, oxidation, and deflation. The surface of the sediments has been lowered by at least 3 feet, and undoubtedly some of the original surface has been lost through deflation. Shorelines of the original lake exist from 15 to 25 feet above the present surface. The lowest level sampled consists of fine sand and silt which grades into clay at 6.5 meters. The clay changes to gray-brown sedimentary peat at 5 meters, which in turn grades into brown fibrous peat at 4 meters. The last type continues to the surface and becomes coarser upward in the profiles. Both profiles are essentially the same with respect to the thickness of the various types of sediments. *Hypnum* moss leaves are present throughout the fibrous peat. A layer of pumice less than an inch thick occurs at 1.75 meters in both profiles. It is not possible to state the source of the pumice because there have been several active volcanoes in the Cascades of Oregon during the post-Pleistocene (Williams 1935, 1941). A bog about 13 miles west of Bend, Oregon, has two layers of pumice, while bogs farther south in the Cascade Range rest on a thick mantle of pumice. The latter evidently came from the eruption of Mount Mazama, which resulted in the formation of the caldera holding Crater Lake.

A third profile of lacustrine sediments was sampled at Onion Flat, about 2 miles northeast of Sherwood, Oregon. This deposit lies about 30 miles north of Lake Labish, and 10 miles south of Portland, at an elevation between 130 and 140 feet above sea level. The peat and other pollen-bearing sediments have accumulated in a former channel of the Tualatin River which rises in the Coast Range to the west and empties into the Willamette River about 2 miles south of Oregon City.[3] Samples were obtained in section 21 of T. 2 S., R. 1 W. The depth of the sediments in the area of sampling is 12 meters. This bog has also been drained and the present surface is not that which existed before drainage. The bog is underlain with sand and gravel which grade into

[2] U. S. Geol. Surv. Topogr. map, Mount Angel Quadrangle, Oregon.

[3] U. S. Geol. Surv. Topogr. map, Oregon City and Tualatin Quadrangles, Oregon.

silt at 12 meters. Fine clay with considerable organic matter present occurs from 12 to 9 meters. The stratum between 9 and 5 meters consists of a gray-brown sedimentary peat, and fibrous peat is present from 5 meters to the surface. The surface sediments seem to have been more oxidized than those at Labish Flats. No definite stratum of pumice or ash is discernible with the naked eye, but volcanic glass is abundant at 0.5 and 0.75 meters. This suggests that a greater thickness of surface sediments has been removed than at Labish Flats, where the pumice layer occurs at 1.75 meters. It is probable that the pumice and glass had the same origin. In none of the profiles, therefore, is the forest succession recorded by tree pollen to the present time. Each year the surface is plowed, so that the upper several inches of peat are not suitable for pollen analysis. The uppermost sample of each profile was obtained from the highest undisturbed horizon.

Peat deposits formed in depressions lying on glaciated sites may be readily dated with their maximum ages. The Willamette Valley, however, was not glaciated during the Pleistocene. The mountain glaciers in the Cascade Range a few miles to the east apparently were of insufficient magnitude to reach the Willamette Valley, nor was the Coast Range to the west glaciated (Fenneman 1931). During the melting of the last stage of the Wisconsin glacier, the Willamette Valley was inundated by backwater from the glacier-swollen Columbia River (Bretz 1919). The water reached a height of several hundred feet above the valley floor as is evidenced by the occurrence of ice-rafted erratics (Allison 1935). This inundation suggests that any peat deposits or other types of lacustrine sediments on the Willamette Valley floor are of postglacial origin. As previously stated, Lake Labish was ponded in an abandoned channel of the Willamette River, probably a distributary of the main stream when it carried a greater volume of water than at present. The Onion Flat sediments were deposited in a former channel of the Tualatin River, although it apparently was not an oxbow left by a meandering stream. It was probably formed when the Tualatin River carried a greater volume of water than the main channel could hold, and spilled over as a distributary to further erode small tributary valleys. These channels must have been occupied by their respective streams when there was considerably more water available from their hydrographic basins than at present. This probably occurred during the early post-Pleistocene when there was heavy precipitation as well as an abundance of meltwater from wasting mountain glaciers. It is hard to say how long these channels were occupied by their respective streams. If they were abandoned soon after the recession of the continental ice sheet to the north and subsidence of glacial water in the Willamette Valley, the sediments of this study had their initiation in early post-Pleistocene. The 12-meter depth of the Onion Flat profile and the forest succession recorded in the lower half suggests that this is probably true.

Twelve meters of sediments, largely organic, are of considerable magnitude, especially in the Willamette Valley where the summers are dry and far from the optimum for the maximum rate of peat deposition. The average depth of 16 post-Pleistocene peat deposits west of the Cascades in Oregon, Washington, and British Columbia is about 31 feet, as shown by profiles in a study by Rigg and Richardson (1938). Most of these deposits lie in regions climatically favorable to rapid peat accumulation. It is realized that climate is not the only factor that controls the rate of organic sedimentation, but peat deposits in the dryer regions east of the Cascades, in Washington, average about 19 feet in depth (Hansen 1941g). The depth of the Onion Flat sediments is almost twice that of Labish Flats. The rate of peat deposition may vary in the same region, but the relative thickness and proportions of clay, sedimentary, and fibrous peat indicate that the former represents a longer period of time, and not merely a more rapid rate of sedimentation. The thickness of sedimentary peat is about 4 meters, whereas that of Lake Labish is only 1 meter. Sedimentary peat probably has the slowest rate of deposition of the several types of sediments present, and such a great difference in its thickness in the two profiles suggest a much earlier origin for the Onion Flat sediments. The thickness of fibrous peat is also much greater in the latter profile. The writer believes that the sediments of Onion Flat were initiated much earlier than those of Lake Labish, and that they date from extremely early post-Pleistocene.

In preparation of the sediments for microscopic examination, the potassium hydrate method was used. One hundred or more pollen grains of indicator species were identified from each level. The number of pollen grains of nonsignificant species was also recorded and listed in the tables. Few or no pollen grains are present in the levels of the bottom meter of the Labish profile. These horizons are not included in the tables or pollen profile diagrams. The identification of the winged conifer pollen was based upon the size range of the pollen of the several species within each genus. This method has been described by the author in several previous papers (Hansen 1941a, 1941b, 1941d). No attempt was made to separate the pollen of Sitka spruce (*Picea sitchensis*) from that of Engelmann spruce (*P. Engelmanni*). It probably consists chiefly of the former, which is abundant along the coast a few miles to the west, while Engelmann spruce is largely confined to the upper slopes of the east side of the Cascade Range. Those species recorded by their pollen as 1.5 per cent or less are listed in the tables as 1 per cent.

FORESTS IN ADJACENT AREAS

The Willamette Valley is from 25 to 30 miles wide and about 125 miles long. It lies within the Humid Transition life zone (Bailey 1936). It is also included in the hemlock-cedar climax of the Coast Forest (Weaver and

Clements 1938), but because of the low summer rainfall the forests are few and scattered. Little or no western hemlock (*Tsuga heterophylla*) and western red cedar (*Thuja plicata*) are present. The valley is intensively cultivated and most of the virgin forests have been removed, but the coniferous forests were never as dense as those of either the Coast and Cascade Ranges (Peck 1941). The principal coniferous species is Douglas fir (*Pseudotsuga taxifolia*) which grows on slopes and better drained areas. Oregon white oak (*Quercus garryana*) is the chief broadleaf tree, and usually occurs in groves. It is difficult to say what the successional relationship is between these two species, because they are often found growing together. The chief other coniferous species present is lowland white fir (*Abies grandis*) which thrives in low areas along the streams or other moist habitats. Farther south scattered specimens of western yellow pine (*Pinus ponderosa*) occur, while in certain localized areas groves of this species exist. On the floodplains of streams and other moist sites grow associes of cottonwood (*Populus trichocarpa*), willow (*Salix lasiandra*), Oregon ash (*Fraxinus oregana*), red alder (*Alnus rubra*), largeleaf maple (*Acer macrophyllum*), and vine maple (*A. circinatum*). It can be seen that the forests of the Willamette Valley are abundant neither in extent nor in the number of species.

The eastern slope of the Coast Range is forested largely with Douglas fir with an occasional hemlock. The summers are apparently too dry for the latter species to thrive. Pollen analysis of a sphagnum bog in the east foothills of the Coast Range and farther to the south shows that hemlock has not been nearly so abundant as Douglas fir during that part of the postglacial period represented by the peat deposit (Hansen 1941a). On the west slope of the Coast Range the precipitation increases and western hemlock becomes more abundant at the expense of Douglas fir. Along the coast is a strip composed largely of Sitka spruce and western hemlock, and along the immediate ocean on the stabilized sand dunes and other sandy soil thrives lodgepole or shore pine (*Pinus contorta*). Pollen analyses of two bogs on the Oregon Coast show that western hemlock and Sitka spruce have been predominant during the post-Pleistocene (Hansen 1941d). On a few of the higher peaks of the Coast Range noble fir (*Abies nobilis*) occurs, while western white pine (*Pinus monticola*) and silver fir (*A. amabilis*) have been recorded at several stations in the northern part. On the western slope of the Cascade Range facing the northern part of the Willamette Valley, the forests are composed chiefly of Douglas fir, with western hemlock increasing in abundance with the altitude. Also at higher elevations thrive western white pine, silver and noble fir, lodgepole pine, western red cedar, and Englemann spruce. The last is not so common on the west as the east slope. Near timberline, mountain hemlock (*Tsuga Mertensiana*), alpine fir (*Abies lasiocarpa*), white-bark pine (*P. albicaulis*), and Alaska cedar (*Chamaecyparis nootkatensis*) flourish.

The presence of pollen in the sediments from all or most of these species justifies the mention of their occurrence in adjacent areas. Less significant species, as far as pollen analysis is concerned, that thrive on favorable sites in the Willamette Valley and adjacent slopes of the Coast and Cascade Mountain Range are *Arbutus menziesii, Castanopsis chrysophylla, Corylus californica, Myrica californica, Cornus occidentalis, Osmaronia cerasiformis, Philadelphus gordonianus, Amelanchier florida, Prunus emarginata, Ceanothus sanguineus, Crataegus douglasii, Symphoricapos albus*, and *Ribes sanguineum.*

Forest Type Maps (1936) designate the Willamette Valley as non-forested and agricultural lands, and the adjacent mountain slopes as Douglas fir types of various size classes. It is also included in the Pacific Douglas fir forest under the classification by Shantz and Zon (1924). Over most of the hemlock-cedar climax west of the Cascades, Douglas fir is a subclimax species that has been able to persist as one of the chief dominants because of periodic fires (Munger 1940). In western Oregon, however, western hemlock is almost absent and Douglas fir is not likely to be replaced by the former even if no periodic fires occur. The climate is too dry. It would be hard to say what the climax vegetation is for the Willamette Valley, but it appears that the climate and edaphic conditions are such as to form a tension area in which Douglas fir and white oak are in equilibrium at present. An increase in summer rainfall probably would permit an increase in Douglas fir as subclimax to western hemlock, while a decrease in the annual precipitation would perhaps cause a trend toward an oak-grassland sere.

The Willamette Valley is designated as having a humid microthermal climate, with a summer deficiency of precipitation (Thornthwaite 1931). The average mean annual rainfall for five stations in the valley, within a radius of 25 miles to both bogs, is 43.5 inches. About 10 per cent of this occurs during May to August, inclusive (Weather Bur., U.S.D.A., 1936). The elevation of these stations ranges from 400 to 57 feet above sea level. At Glenora, in the northern part of the Coast Range, and less than 50 miles from Onion Flat, the mean annual precipitation is 130 inches. At Government Camp on Mt. Hood, at an elevation of 3800 feet and about 60 miles from either peat deposit, the annual mean precipitation is about 85 inches. Thus the Willamette Valley is bordered by areas with very moist climate, which has undoubtedly influenced the pollen record of the forest trees in the peat profiles. The general direction of the wind during the period of anthesis is westerly, which suggests that the forests of the Coast Range are more strongly represented than those of the Cascades. This is also somewhat corroborated in the pollen profiles themselves.

FOREST SUCCESSION

There is no reason to assume that the Willamette Valley, the Coast Range,

and unglaciated parts of the Cascades were not forested during the Pleistocene when areas to the north were covered with ice. Forests grow to the edge of mountain glaciers at present, and even upon certain glaciers in Alaska (Washburn 1935). The climate of the Pleistocene during continental glaciation, however, may have been more severe, but it was probably sufficiently mild in western Oregon to support forests. The forests that existed within range of pollen dispersal to the site of Onion Flat during the time represented by the lower 6 meters of sediments were composed almost entirely of lodgepole pine, Sitka spruce, and lowland white fir (fig. 1). Lodgepole pine

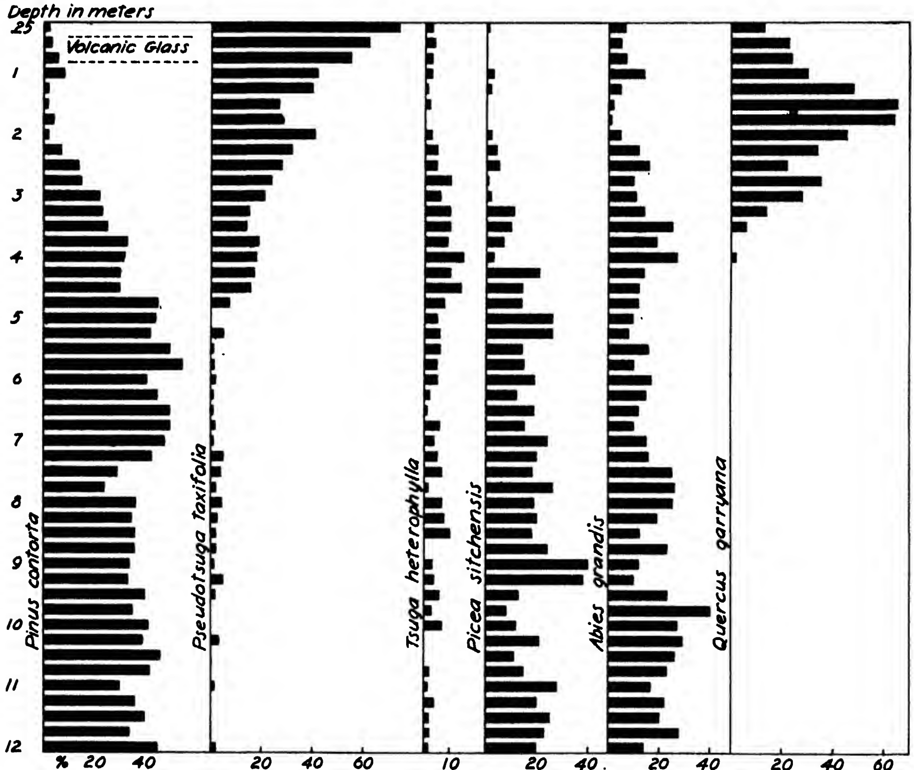

Fig. 1. Pollen Profiles. Onion Flat.

proportions fluctuate from 24 to 50 per cent with an average of about 40 per cent. Sitka spruce fluctuates from 8 to 40 per cent and lowland fir varies from 10 to 40 per cent. While the pollen percentages of lodgepole pine run higher than those of spruce and fir, it does not necessarily mean that it was predominant, since lodgepole sheds a greater quantity of pollen than Sitka spruce, and probably more than lowland white fir. It is interesting to note that lodgepole pine and Sitka spruce are entirely absent from the Willamette Valley and the east slope of the Coast Range at present. These two species may be over-represented by their pollen because of its transportation by

water. The Tualatin River rises in the Coast Range so that its drainage area is in proximity to the spruce and lodgepole forests of the Pacific Coast. During high water in the spring the Tualatin River may have spilled over into its former channel occupied by the accumulating Onion Flat sediments. Pollen from the coast forests reached the streams and were carried into the lake and incorporated with the sediments. The relatively low proportions of western hemlock pollen in these horizons, however, do not substantiate this theory, or perhaps hemlock was not as abundant during early post-Pleistocene as it is today on the coast. It was apparently predominant during most of the post-Pleistocene on the Oregon Coast (Hansen 1941d).

Lodgepole pine continues its predominance upward in the Onion Flat profile to the 3.75 meter level. It is also recorded by its pollen as the principal species with fir and spruce in the lower 2.5 meters of the Lake Labish profiles (figs. 2, 3). As previously stated, the greater depth of the Onion Flat profile

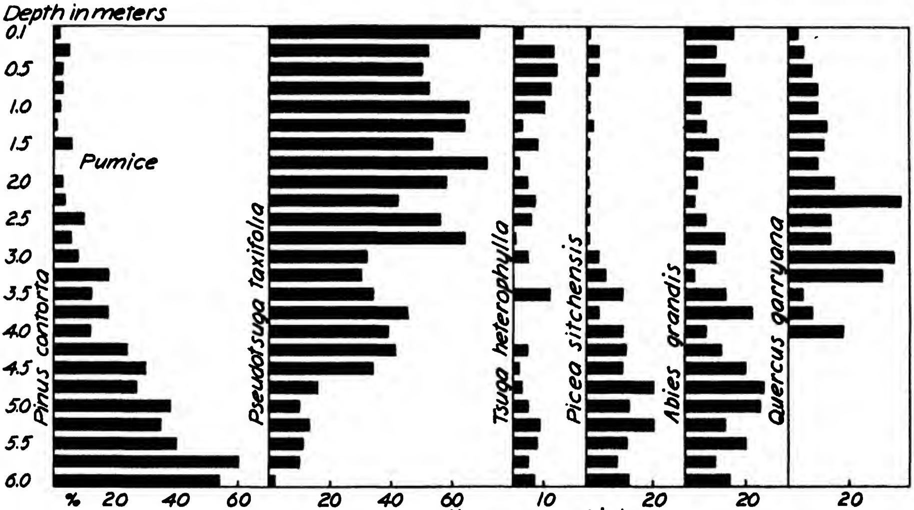

Fig. 2. Pollen Profiles. Lake Labish. No. 1.

indicates a longer period of time for its sedimentation than that of Lake Labish. It seems possible that the upper 7 meters of the former are contemporaneous with the entire Lake Labish profiles. They also are 7 meters deep, but, as stated above, the lower meter is practically devoid of pollen. The pollen spectra of the principal species recorded correlate remarkably well in the upper 6 meters of all three profiles, which substantiates the theory of their synchronism. The predominance of lodgepole pine for such a long period of time as portrayed in the Onion Flat profile is significant. In several other bogs analyzed for their fossil pollen that lie beyond the glaciated region, lodgepole pine is not recorded as the predominant species in adjacent areas (Hansen 1939c, 1941a, 1941c, 1941d, 1941f). This suggests that the

adjacent forests had reached a climax when the lowest pollen-bearing sediments were deposited or had remained more or less stabilized during the glacial period, or at least during the latter part. The forests apparently had not been destroyed during glaciation nor had conditions favorable for an invasion by lodgepole developed. This was true even along the Oregon Coast where lodgepole pine thrives at present (Hansen 1941d). In a bog farther south in the Willamette Valley, in the east foothills of the Coast Range, Douglas fir is recorded as having been predominant during the entire period represented by the profile (Hansen 1941a). Either the sediments do not record all of the postglacial, or lodgepole was not abundant in this region

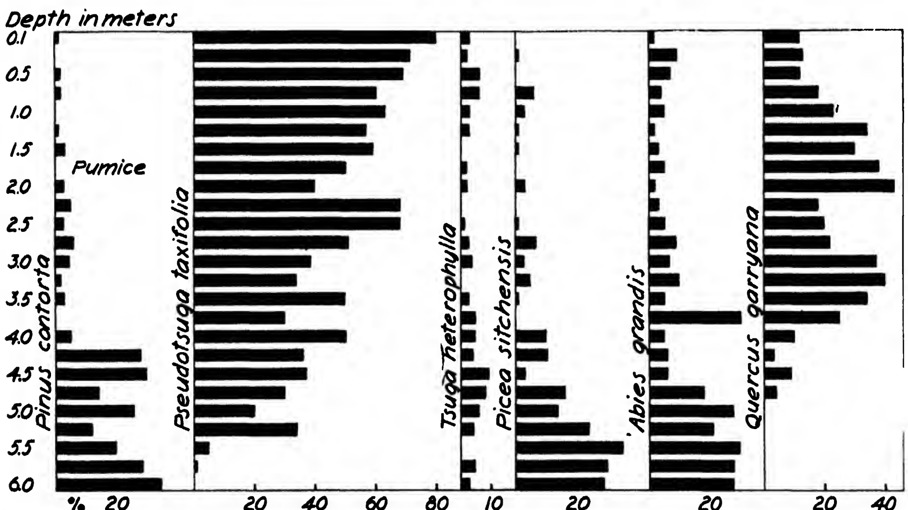

Fig. 3. Pollen Profiles. Lake Labish. No. 2.

at any time during the post-Pleistocene. In other peat deposits that lie within the glaciated region, lodgepole pine was the predominant species recorded in the bottom sediments. As these sediments are usually sand and silt, it seems probable that pioneer postglacial forest succession was recorded. It is believed that lodgepole pine flourished near the ice-front because of lack of competition of other species for which the climatic and edaphic conditions were unfavorable. As the environment became moderated and somewhat stabilized, other species invaded and replaced lodgepole because of its greater intolerance for shade and shorter life span. This was evidently true both east and west of the Cascade Range in Washington (Hansen 1938, 1939a, 1939b, 1940a, 1940b, 1941b, 1941e). The comparative absence of Douglas fir and western hemlock in the lower horizons of all profiles suggests that the edaphic and climatic conditions were unfavorable for these species. The high proportions of Sitka spruce and lowland white fir, both moisture-loving species, suggests that the climate was very wet. Heavy precipitation and melting of

the glaciers in the Cascades may have resulted in erosion in the hills and inundation in the valley, with constantly changing edaphic and topographic conditions. Lodgepole pine was able to survive in abundance because of its ability to live under an adverse climate and unstable edaphic conditions, and its early seed-bearing age. The latter characteristic permits it to migrate more readily and re-establish itself under changing edaphic and topographic conditions than the other species. This is evidenced by its invasion of pumice-covered areas in the Cascades of southern Oregon, burns, sphagnum bogs, and sand dunes along the Pacific Coast. The pollen percentages of lodgepole pine generally decline upward in all profiles from their maxima in the lower levels and become negligible in the upper horizons (figs. 1, 2, 3). Sitka spruce and lowland white fir are most abundantly recorded in the lower levels of the Labish profiles as well as in the other. In one of the former they both show higher proportions at several levels than lodgepole pine (fig. 3). Spruce is the first to show a decline and it is recorded by negligible percentages in the upper 3 meters of all profiles. Lowland white fir maintains higher proportions in the higher horizons, indicating that conditions remained more favorable nearer to the present time for this species than for spruce. Lowland white fir is common in the Willamette Valley today, and is recorded to 16 per cent in the top level of one of the Labish profiles (fig. 2).

Douglas fir is recorded by its pollen in low proportions in the lower 7 meters of the Onion Flat profile with a maximum of 5 per cent at 3 levels (fig. 1). It likewise is represented by slight percentages in the lower horizons of the Lake Labish profiles (figs. 2, 3). This is consistent with its trend in the Puget Sound region (Hansen 1938, 1940a, 1941b). It increases sharply from 4.75 meters in the Onion Flat profile and from 5 and 5.5 meters in the others, to attain 74, 69, and 80 per cent respectively in the uppermost level. In its general increment upward toward the top, Douglas fir shows a number of fluctuations of considerable magnitude. The greatest of these variations occurs conversely with those in the pollen spectra of Oregon white oak and will be discussed later. Bogs located within the hemlock-cedar climax of the Coast Forest in the Puget Lowland of western Washington show Douglas fir as rapidly replacing the pioneer forests of lodgepole and western white pine (Hansen 1938, 1940a, 1941b). It was in turn replaced more gradually by western hemlock to some extent, but was able to persist in equal abundance with the latter, owing to periodic fires that interrupted forest succession toward the hemlock-cedar climax. It did not, however, attain so high proportions as in the profiles of this study. In eastern Washington, Douglas fir has played a minor part in postglacial succession because of the dry climate (Hansen 1939b, 1940b, 1941f). On the Pacific Coast of Oregon and on the west side of the Olympic Peninsula it also has been unimportant in the forest complex during the post-Pleistocene (Hansen 1941c, 1941d). Douglas fir has

been predominant on the east slope of the Coast Range in west central Oregon during most of the postglacial period (Hansen 1941a).

The pollen of white oak does not make its appearance in the lower levels of the profiles. In the Onion Flat profile it is first recorded at 4 meters by 1 per cent, and in the others at 4.57 and 4.75 meters with 10 and 4 per cent respectively (figs. 1, 2, 3). The fact that this species is not recorded by its pollen in the lower levels in the Onion Flat profile is further evidence that this profile represents a much longer period of time. Oak soon increases sharply after its initial appearance, being recorded as 35 per cent at 2.75 meters in the Onion Flat profile, and 35 and 40 per cent at 3 and 3.25 meters respectively in the Lake Labish sediments. In the latter, Douglas fir shows abrupt declines at these same levels. Oak then precipitately declines to 22 per cent in the Onion Flat profile at 2.5 meters, and to 14 and 18 per cent at 2.5 and 2.25 meters respectively in the other two. It again increases to 65 per cent in the former at 1.5 meters, and to 37 and 43 per cent at 2.25 and 2 meters respectively in the Lake Labish sediments. These are the highest proportions attained in the white oak spectra. That these maxima are all approximately synchronous is denoted by the occurrence of the pumice layer immediately above in the Labish profiles and slightly higher in the other (figs. 1, 2, 3). The general trend of Douglas fir increment is again interrupted at the oak maxima, showing a decline to less than the oak proportions in the Onion Flat and one of the Labish profiles. It should be noted that this increase and decrease of oak and Douglas fir respectively are not merely relative. There is an actual decrease in the frequency of Douglas fir pollen at these horizons. White oak declines from these maxima, and is recorded by 13, 3, and 12 per cent in the uppermost horizons. In a bog near Tacoma, Washington, located on the Tacoma "prairies," oak is recorded by its pollen to as high as 14 per cent in the middle horizons of a 10.5 meter peat profile (Hansen 1938). This area is characterized by scattered groves of white oak on the gravelly soil of the outwash plain from the Vashon glacier of the Puget Sound region. The oak maximum in this bog may be synchronous with those of this study. It seems possible that white oak is under-represented, because of the vast areas on either side of the Willamette Valley forested with Douglas fir, while oak is and probably always has been confined to the valley. Also from the writer's observations it seems probable that oak does not produce as much pollen as Douglas fir.

Western white pine is recorded by its pollen to higher proportions in the lower levels of all profiles and declines to only a trace near the top (tables). This trend is consistent with that of most peat profiles west of the Cascade Range. Western hemlock is the most abundantly recorded of the other conifers. In the Onion Flat profile it attains its greatest abundance in the middle horizons, as it does in one of the others. In the third, it shows its

TABLE 1

Percentages of Fossil Pollen. Onion Flat Profile

Depth in meters	*Pinus contorta*	*P. monticola*	*P. ponderosa*	*Pseudotsuga taxifolia*	*Tsuga heterophylla*	*T. mertensiana*	*Picea sitchensis*	*Abies grandis*	*A. nobilis*	*A. lasiocarpa*	*Thuja plicata*	*Quercus garryana*	*Pinus* spp.*	*Abies* spp.*	*Alnus**	*Salix**	Cyperaceae*	*Typha**	*Nymphozanthus**
0.25	2			74	3		1	7				13			5	156	4	3	
0.5	3	1	2	62	4			5				23	1	1	3	198			
0.75	5	2	4	55	3			7				24	1	1	4	94			
1.0	8	1		42	3		3	15				30			2	208			
1.25	2	2		40	1		2	5				48	2			35			
1.5	2	1		27	2			2			1	65	1		5	27	7	1	
1.75	4	2		29				1				64	1			15		1	
2.0	2	1	1	41	3		2	5				45	1		2	12	3		
2.25	7	5		32	5		4	12			1	34	3		1	10		1	
2.5	14	3	7	28	5		5	16				22	3		1	3			
2.75	15	4		24	10	1	1	10				35			3	10			
3.0	22	2		21	6		2	11			8	28	3	1	1	4	6		
3.25	23	11		15	10	2	11	14				14	2			4	1		1
3.5	25		4	14	13		10	25	3			6	7			2	2	1	1
3.75	33	2	8	19	9	1	7	19			2		4		4	2	3	23	26
4.0	32	2	2	18	15		3	27				1	3	3	3	4	5	60	32
4.25	30	2		17	10		21	14		2	4		9	3	12	5		3	4
4.5	30	10		16	14	4	14	12					4	2	1	2		3	
4.75	45	8	2	7	8	1	14	12	1	2			8	1		9	3	2	
5.0	44	7			5	3	26	10	2	3			3		1	12	3	3	2
5.25	42	10		5	6	3	26	8					7	3	1		5	4	
5.5	50	5	1	1	6	2	14	16	1	4			9			1	8	4	
5.75	55	10	1	1	5		15	10		3			11	1		6		2	
6.0	41	10		2	5	4	19	17		2			14	3		4	1	2	
6.25	45	20	2	1	2	1	12	15	1	1			15	1		5	1	5	
6.5	50	7	3	1	1	4	19	12	1	2			7	2		3		7	
6.75	50	10	2	2	6	5	15	10					12	2	3	1	5	4	
7.0	48	3		1	4	3	24	15	1	1			7	1		5	7	8	
7.25	43		1	5	5	5	20	16	3	2			9	4		9	11	5	
7.5	29	5	4	4	7	8	18	25					11	3		3		4	
7.75	24	8	4	2	1	7	26	26	1	1			5	2		2	8		
8.0	36	6	2	4	7	2	19	25					6	3			2		
8.25	35	13		3	8	1	20	19	1				7	1		1	2		
8.5	36	12	2	2	10	6	18	12		2			2	1		7	5	1	
8.75	36	6	2	2		2	24	23		5			6	1		4			
9.0	34	6		1	3	4	40	12					17	5		3	1	1	3
9.25	33	4		5	4	4	38	10	1	1			12	2		1	2	1	
9.5	40	10	2	2	6	4	13	23					6	3		7	2	4	
9.75	35	8			3	2	8	40	4				7	1		2			
10.0	41	13			7		12	27					9	6		1	1		
10.25	39	6		3		2	21	29					14	2		5			
10.5	46	11	2			3	11	26	1				1	4	1		1	1	
10.75	42	12	4		2	1	15	23	1				11	3		4	2		
11.0	30	16	5	1	1	1	28	17	1				7	1		2			
11.25	36	8	6		4	4	20	22					5	2		1			
11.5	40	10			2	2	25	20		1			7	2	1		5		
11.75	34	13			2		23	28					5	1	2		4		
12.0	45	14		2	2	2	20	14	1				12	3			1	2	

* Number of pollen grains; not computed in the percentages.

highest frequencies in the lower two-thirds. Its maximum is 15 per cent in any of the profiles (tables). The climate has probably been too dry for this species in the Willamette Valley. In the Puget Lowland it partially replaced Douglas fir, to become equally abundant during the latter half of the post-glacial. Mountain hemlock is appreciably recorded in the lower two-thirds of the Onion Flat profile, and to only a trace in a few levels of the others (tables 1, 2, 3). Neither western nor mountain hemlock shows a trend that correlates with the spectra of the other species. Pollen of the latter probably drifted down from higher altitudes in the Cascades, or less probably from the northern part of the Coast Range. Western yellow pine is recorded sporadically in the lower horizons and more consistently in the higher levels. This trend differs from that in the bog farther south in the Willamette Valley, where it is more abundantly recorded in the lower levels (Hansen

TABLE 2

Percentages of Fossil Pollen. Labish Profile No. 1

Depth in meters	*Pinus contorta*	*P. monticola*	*P. ponderosa*	*Pseudotsuga taxifolia*	*Tsuga heterophylla*	*T. mertensiana*	*Picea sitchensis*	*Abies grandis*	*A. nobilis*	*Thuja plicata*	*Quercus garryana*	*Pinus* spp.*	*Abies* spp.*	*Alnus**	*Salix**	Cyperaceae*	*Typha**	*Nymphozanthus**
0.10	2	...	5	69	3	...	1	16	1	...	3	1	3	3	...	1	...	...
0.25	5	...	9	52	13	...	4	10	1	1	5	2	2	2	15		...	...
0.5	3	...	8	50	14	...	4	13	...	...	8		1	6	43	6	1	...
0.75	3	...	7	52	12		1	15	...	...	10	3	3	1	32	6	...	...
1.0	2	...	7	65	10	...	1	5		...	10			1	15	1	1	1
1.25	1	2	8	64	3	...	2	7	...	...	13	1	2	8	117	3	...	...
1.5	6	...	8	53	8	1	1	11	...	...	12	...	...	1	12		2	2
1.75	1	...	9	71	2	...	1	6	...	...	10	2	1	5	...	7	1	...
2.0	3	1	13	58	5	...	1	4	...	...	15	...	...	...	10	1	6	1
2.25	4	...	5	42	7	...	1	3	...	1	37	1	1	1	3	1	3	1
2.5	10	...	6	56	6	...	1	7	...	...	14	2	...		15	2	2	...
2.75	6	1		64	1	...	1	13	...	...	14	1	2	3	10	3	7	...
3,0	8	4		32	5		4	10	2	...	35	1	1	7	3	9	3	2
3.25	18	...	12	30	...	...	6	3	...	...	31	5	1	...	8	1	...	1
3.5	12	8	2	34	12	...	12	13	2	...	5	3	1	1	39	7	...	...
3.75	18	...		46	...	...	4	22	2	...	8	2	3	6	23	3	...	2
4.0	12	...	12	39		...	12	7	...	...	18	4	2	...	25	5	1	1
4.25	24	5	...	41	5	...	13	12	...	...	...	6	1	5	19	2	...	3
4.5	30	2	...	34	2	...	12	20	...	...	...	7	7	...	21	...	...	...
4.75	27	3	...	16	3	...	22	26	3	...	...	3	6	1	15	1	...	2
5.0	38	1	...	10	7	...	14	25	3	2	...	5	5		12	3	...	...
5.25	35	7	...	13	9	...	22	13	1	...	...	7	4	4	7	3	...	...
5.5	40	4	...	11	8	...	13	20	2	2	...	12	3	...	5	...	...	1
5.75	60	5	...	10	5	...	10	10	...	...	...	15	1	2	3	1	...	...
6.0	54	6	...	2	7	...	14	15	1	...	...	17	3	3	...	...	...	...

* Number of pollen grains, not computed in the percentages.

1941a). Its trend in this study is more logical, however, because it is the most xerophytic of the conifers, and should have increased during later postglacial time, if a drier climate prevailed. Other species recorded by their pollen are willow, sedge, yellow pond lily, cat-tail, alder, hazel, ash, and maple (tables 1, 2, 3). The first four record the progress of hydrarch succession. The scarcity of alder pollen is unusual because it is generally abundant in most peat profiles in the Pacific Northwest. In some cases alder seems to be correlated with Douglas fir, which suggests that it invades low, damp areas that have been denuded by fire (Hansen 1941a).

CLIMATIC CONSIDERATIONS

In this study the pollen spectra of Sitka spruce, lowland white fir, Douglas fir, and white oak are the best indicators of climatic trends. Spruce is an

TABLE 3

Percentages of Fossil Pollen. Labish Profile No. 2

Depth in meters	*Pinus contorta*	*P. monticola*	*P. ponderosa*	*Pseudotsuga tarifolia*	*Tsuga heterophylla*	*T. mertensiana*	*Picea sitchensis*	*Abies grandis*	*A. nobilis*	*Quercus garryana*	*Pinus* spp.*	*Abies* spp.*	*Alnus**	*Salix**	Cyperaceae*	*Typha**	*Nymphozanthus**
0.10	1		2	80	3			2		12	1		2	3		3	
0.25			4	71	2		1	9		13		2	3	1		4	
0.50	2		4	69	6			7		12	2	1		2			
0.75	2		4	60	6		6	4		18	1		1	1			
1.0			3	63	3		3	5		23		1		9			2
1.25	1		2	57	3		1	2		34			7	7		2	1
1.5	3		4	59			1	3		30	1		4	12			
1.75			5	50	2			5		38			2	15	2		1
2.0	3		6	40	2		3	2	1	43	2	1		21	5		15
2.25	5		5	68				3	1	18	1		1	14	4	5	1
2.5	3		2	68	1		1	5		20				18		2	5
2.75	6	1	1	51	3		7	9		22	3	1	1	32		1	1
3.0	5	2	3	39	4		3	7		37			1	27	8	7	3
3.25	2	5		34	1		5	10	3	40	1	1		48	9	3	2
3.5	3		2	50	3		1	5	2	34	1	3	3	53	1	4	1
3.75			10	30	5			30		25	2		2	17	4	1	
4.0	5	4	1	50	5		10	5	10	10	1	2	7	9	2	1	
4.25	28	13		36	4		10	6		3	5			12	7		
4.5	30	6		37	9		3	6		9	8	1	5	42	4	1	
4.75	14	8		30	8		16	18	2	4	9	2		112		2	
5.0	26	6		20	6		14	28			11	3	1	59	5		
5.25	12			34	4	2	24	20	4		5	1		216	3		
5.5	20	10		5			35	30			7	3	2		1		1
5.75	29	7		1	5		30	28			9	3	3				
6.0	35	5			3		29	28			5	2					

Number of pollen grains, not computed in the percentages.

indicator of extremely moist conditions, whereas white oak thrives under the driest climate. Lowland white fir is next to spruce in its moisture requirements, and Douglas fir will survive under drier conditions than either spruce or lowland white fir, but not so xeric as oak. Lodgepole pine is a poor climatic indicator because of its wide climatic and geographic range. The predominance of its pollen in the lower levels of the Pacific Northwest peat profiles marks its invasion of primary areas before the edaphic and physiographic conditions had been stabilized and sufficiently moderated to permit the entrance of other species more tolerant of shade and with a longer life span to replace it. Western hemlock thrives under a moist climate, but it is too sparsely represented in the peat profiles of this study to serve as a climatic indicator. Its low pollen frequency, however, in itself denotes that too dry conditions prevailed for its existence in the Willamette Valley, at least during the latter two-thirds of the post-Pleistocene. Western white pine is indicative of a cool and subhumid climate, and mountain hemlock marks at least a cool climate. Yellow pine is almost as xerophytic as white oak.

In the Puget Lowland of western Washington, postglacial forest succession has probably been more a result of competition than due to climatic change. This also seems to have been true of the Oregon Coast and the west side of the Olympic Peninsula (Hansen 1941c, 1941d). In drier regions where the annual precipitation or summer rainfall is at a critical minimum, slight changes may influence forest succession over a period of time. The greater response of tree growth in dry areas is shown by comparative studies of radial increment in trees living in wet and dry climates (Douglass 1936). In dry climates, slight variations in the annual precipitation are readily reflected by the width of the annual rings, whereas in moister climates the ring width may vary only slightly from year to year, in spite of appreciable differences in the annual rainfall. It is believed that the small amount of summer rainfall in the Willamette Valley is responsible for recording more definite postglacial climatic trends than elsewhere west of the Cascade Range. High percentages of Sitka spruce and lowland white fir pollen in the lower half of the Onion Flat profile mark a wet climate during the first third of the post-Pleistocene. A continuation of proportions of appreciable but lesser magnitude upward in this profile, as well as in the contemporaneous horizons of the lower Lake Labish profiles, marks a persistence of the wet climate for some time longer. The scarcity or absence of spruce pollen in the upper horizons of all profiles and the absence of this species east of the Coast Range at present, substantiates this interpretation. The predominance of lodgepole pine as denoted in the lower strata marks the presence of unstable soil and physiographic conditions, possibly caused by increased surface water from melting glaciers in the Cascades and greater precipitation in the valley. This probably resulted in considerable erosion, deposition,

inundation, emergence, and other changing environmental conditions under which trees requiring 25 years or more to reach seed-bearing age could not persist. The invasion and rapid increase in Douglas fir signify a drier climate and more stabilized edaphic and physiographic conditions as the postglacial period progressed. Instead of the entrance and development of western hemlock to attain equal abundance with Douglas fir, as occurred in the Puget Lowland, white oak invaded the valley and rapidly increased in its extent to supersede Douglas fir at some levels in its pollen proportions. This significant increase at the expense of Douglas fir marks further desiccation of the climate. The maximum of this trend was apparently reached just prior to the eruption of one of the Cascade volcanoes and the deposition of pumice in the accumulating sediments. This period of maximum dryness, as portrayed by the influx of oak, is not corroborated by a simultaneous invasion of grasses and composites, such as occurred on the Tacoma "prairies" just south of Puget Sound (Hansen 1938). A decline in the pollen percentages of oak and a resumption of Douglas fir predominace to the uppermost horizon may mark a cooler and moister climate in more recent time.

A general decrease in moisture during the middle third of the post-Pleistocene is suggested by most of the pollen spectra of Pacific Northwest peat profiles, with the exception of those in proximity to Puget Sound. Some peat deposits, however, record more definitely a dry, warm period, succeeded by a slight increase in moisture in more recent time. In a bog near Spokane, Washington, located in a western yellow pine climax, a sharp increase in the proportions of grass, chenopod, and composite pollen about one-third the way upward in the profile signifies the occurrence of a hot, dry climate (Hansen 1939b). These species were later replaced by forests consisting largely of yellow pine, suggesting a return of a moister and cooler climate, which persisted to the present time. Lake sediments in the Upper Sonoran life zone of east central Washington record relative trends of forest and grassland succession that depict a gradual drying and warming to a maximum, followed by some cooling and greater precipitation (Hansen 1941e). Pollen analyses of four profiles of lake sediments from Lower Klamath Lake of southern Oregon and northern California also denote the development of a xeric period, followed with an increase in moisture (Hansen 1941g). The evidence offered by the pollen profiles is further corroborated by the occurrence of artifacts underlying from 6 to 8 feet of fibrous peat (Cressman 1940). The wide distribution of the artifacts over hundreds of square miles of the exposed fossil lake bed marks the drying up of the lake and the occupancy of the lake bed by early man between 7500 and 4000 years ago (Antevs 1940). The salinity of certain lakes in the Great Basin is also supporting evidence for the occurrence of a xeric period during the postglacial. The present salinity of these lakes is such as to have required not over 4000

years for its development (Antevs 1938). The lakes had their origin in the Pleistocene, dried up during the post-Pleistocene, and their precipitated salts were removed by deflation or buried. The present lakes were reformed in their freshened basins about 4000 years ago (Antevs 1940). The drying and reforming of these lakes seem to be closely correlated with the disappearance and rebirth of most of the western mountain glaciers. Evidence shows that the existing glaciers in the Sierras are not remnants of the Pleistocene glaciers, but those that came into existence only a few thousand years ago (Matthes 1939). Their recession and readvance apparently have been more or less synchronous with the drying and rebirth of the lakes of the Great Basin. Pollen profiles from eastern North America also designate a drying and warming to a maximum, followed by a somewhat cooler and moister climate (Smith 1940).

SUMMARY

Three profiles of lake sediments in the Lower Willamette Valley of western Oregon record an interesting and significant trend of post-Pleistocene forest succession by the forest tree pollen recorded therein. The depth and pollen spectra of the Onion Flat profile suggest that sedimentation was initiated in early post-Pleistocene, and that it records adjacent forest succession from the Pleistocene almost to the present. An unknown thickness of the upper sediments has been removed in all profiles, due to drainage and cultivation. The Onion Flat profile probably represents a longer period of time for its deposition than the Lake Labish profiles.

The earliest recorded forests of the Willamette Valley and adjacent slopes of the Coast and Cascade Ranges consisted largely of lodgepole pine, Sitka spruce, and lowland white fir. This is extremely significant, because the first two species are seemingly absent from the Willamette Valley at present. These initial postglacial forests were slowly replaced by Douglas fir, which in turn was replaced to some extent by white oak. Douglas fir shows a general increase upward in the profiles with white oak, which supersedes the former in the upper levels of the profiles. Oak declines to the top after it attained its maximum, and Douglas fir resumed its predominance to the uppermost level.

Climatically this trend of forest succession denotes a long period of moist conditions with considerable instability of the edaphic and physiographic conditions. The latter conditions are suggested by the persistence of lodgepole pine as indicated by the presence of its pollen in abundance in the lower two-thirds of the Onion Flat profile. As the influence of recent glaciation waned, the climate became warmer and drier, as is evidenced by the invasion of Douglas fir, followed by that of white oak. The xeric period reached its maximum during the period of oak predominance. The climate

became moister and cooler during the latter part of the postglacial. The small amount of western hemlock pollen in the profiles indicates that the initial edaphic and physiographic conditions were too rigorous for its existence, and later the climate became too dry for its development to any great extent. The evidence for this climatic trend is more definite in this study than that offered by other pollen profiles west of the Cascades. Evidence for a similar dry period is present in peat profiles east of the Cascades in Washington and Oregon. These climatic interpretations are also supported by anthropological and geological studies in the Great Basin.

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VEGETATIONAL STUDIES IN AREAS OF SEDIMENTATION IN THE BONNET CARRÉ FLOODWAY

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INTRODUCTION

The City of New Orleans was founded in 1718 "to secure the river and Louisiana against covetous Spain and England" (La. Writers Project, 1941). Ever since its founding, the city has been subject to the threat of recurrent floods. No comprehensive plan of levee construction, however, was begun until 1828, when a severe flood caused legislative action by the State of Louisiana (Russell 1936). Although much construction had been accomplished by 1882, the great flood of that year, sweeping through 284 crevasses and washing away 56 miles of levee, set the stage for a concerted campaign by the State and Federal governments to control the river. The levee system, however, again proved inadequate in 1927, at which time the levee at Caernarvon below New Orleans was dynamited to relieve flood pressure on the city. This resulted in further flood control projects such as storage reservoirs, cut-offs and artificial floodways.

One of these artificial floodways is the Bonnet Carré Floodway, located at a point some 20 miles upstream from the city of New Orleans. It has been constructed in an area where several natural crevasses have occurred. The most extensive of these, just above the site of the present floodway, was created when the artificial levee was breached on April 11, 1874. This crevasse remained open for almost nine years and built up an extensive area of alternating ridges and swales. This type of land surface is known as "Crevasse topography" and is plainly evident between the present floodway and the town of La Place.

The Bonnet Carré Floodway consists of a one-and-one-half-mile concrete weir (spillway), which fronts the river, and two guide levees which extend in a Northeasterly direction for a distance of about five-and-one-half miles to Lake Pontchartrain. It was first utilized in the winter of 1937 to relieve the pressure of the greatest controlled flood in the history of the Mississippi River. The spillway was opened on January 29, 1937, and remained open until March 17, 1937 (fig. 1). The passage of the flood waters resulted in

[1] The writers are indebted to the New Orleans District Office of the United States Army Engineers for permission to make studies in the floodway and for data concerning sedimentation. To Faith Pennebaker Mackaness, Tulane University, and Thomas F. Hall, Tennessee Valley Authority, for assistance on several field trips the authors express their gratitude. They are indebted also to Mrs. Agnes Chase who identified several grasses and to Dr. Henry K. Svenson for the identification of a number of perplexing sedges.

considerable deposition of alluvium. The sediments were deposited in a striking pattern of irregular sand ridges or dunes up to 14 feet in height. Since several natural crevasses have occurred in the immediate vicinity these deposits are superimposed, in part, on "crevasse topography" of former natural diversion channels.

FIG. 1. The spillway (right) was opened on January 27, 1937, to relieve flood pressure on the City of New Orleans. (Floodway at left.)

DISTRIBUTION AND COMPOSITION OF SEDIMENTS

The bulk of the alluvium was deposited near the spillway, because of the decreased carrying power of the flood waters occasioned by the very great change in the direction of the flow. Furthermore, low-growing groups of trees, shrubs, and vines augmented the deposit and increased the height of the ridges, especially on the lakeward side of the masses of vegetation.

Although the dunes included fine sand, silt, and clay, the coarser sediments predominated. Data supplied by the United States Army Engineers Office reveal the fact that the percentage of finer sediments increased gradually with the distance from the weir. It should be pointed out, however, that the sediments in areas of slight deposition, even though relatively close to the spillway, were much finer than the alluvium of the dunes and swales.

With the composition of the sediments in mind, stations were selected relatively near (1000–1500 feet) the spillway, adjacent to the airline highway (about 9000 feet from the spillway) and in an area of slight deposition

on the lakeward side of the airline highway. The components of the soil, as determined from surface samples, at these stations are much alike except at Station 3, which was located in an area of slight deposition.

	Per cent		
	Sand	Silt	Clay
Station 1 (near spillway)	93.3	3.4	3.3
Station 2 (near highway)	89.9	3.3	6.8
Station 3 (lakeward from highway)	2.9	69.6	27.5

Determinations of water content were made at all stations in May, August, and October, 1937. The content was least in August and highest in the month of October but was ample throughout the growing season. The unusually high soil moisture in October is in accord with the abnormal rainfall for that month (25.11 inches) which was 21.81 inches more than the long term average and the highest precipitation for any one month in the history of the New Orleans Weather Bureau. As might be anticipated, water content increased with the depth of the sample. Furthermore the percentage of water was greater at the same sample depth in the swales than on the ridges.

Fourteen determinations of the pH of the sediments were made by a La Motte-Morgan indicator set. These tests gave a pH range of 7.6 to 8.4 but did not indicate any correlation with topography, water content, or vegetation.

ORIGINAL VEGETATION

The original land surface in the floodway slopes gradually from the Mississippi River to Lake Pontchartrain about five-and-one-half miles to the northeast. The frontlands along the river are composed of coarser sediments and are better drained than the backlands. It is probable that the frontlands were clothed originally with a hardwood forest in which water oak, live oak, and hackberry were the predominant species. The much more extensive backlands were occupied by a swamp forest in which bald cypress and tupelo gum were the important dominants.

At the time the floodway was completed in 1935 the frontlands were largely in cultivation. But there were scattered water oaks, live oaks, hackberries, pecans, and sycamores, as well as numerous specimens of rough-leaved dogwood, poison ivy, pepper-vine, supple-jack, and trumpet-vine. The swamp forest, although completely cut over in the past, still presented a true forest aspect, with a fairly continuous stand of bald cypress, tupelo gum, and swamp maple.

EFFECT OF FLOODWAY OPERATIONS ON PREEXISTING VEGETATION

As far as could be ascertained the flood waters per se produced no observable deleterious effect on the former vegetation in the floodway. This was

true in spite of the fact that the water covered the soil to a considerable depth (several feet) for a period of more than 46 days (the period during which the spillway was open). The answer probably lies in the fact that the spillway was opened on January 29 before much plant activity had started. Furthermore, the relatively cold water in the floodway undoubtedly prolonged the dormancy of many species of woody plants.

On the other hand the alluvial deposits, especially those over one foot in thickness, killed nearly all herbaceous plants, a considerable number of shrubs, vines, and seedling trees, and a few sapling trees. In order to determine the percentage destruction of woody plants, ten temporary quadrats were established and studied in June and August. Individual shoots that projected through the alluvium were listed according to species, their diameter at the ground level in inches (d.g.l.) and their state of health. Since it was often impossible to determine whether a shoot represented an individual or merely a branch of a plant, each shoot was given equal rank in the averages. The shoots were listed as healthy, unhealthy, or dead. Typically, the leaves of unhealthy plants were yellowed or were browned from the edges inward. In the absence of leaves, the shoots were cut to determine whether they were living or dead.

On the whole, the healthy shoots far outnumbered the unhealthy and dead specimens in June. Of the 465 individual shoots examined in all habitats, 282 were healthy, 87 were sickly, and 96 were dead. On the ridges the proportion of healthy shoots was somewhat lower than it was in the swales. This suggests that the destruction varies directly with the thickness of the alluvium.

It was also found that the health of the woody plants varied directly with the d.g.l. of the shoots. This is well illustrated in the following table of four of the common species:

Species	Average d.g.l. (inches) Healthy	Dead
Quercus virginiana[2]	1.94	0.40
Celtis mississippiensis	2.18	1.25
Hicoria pecan	2.16	0.40
Svida asperifolia	1.79	0.49

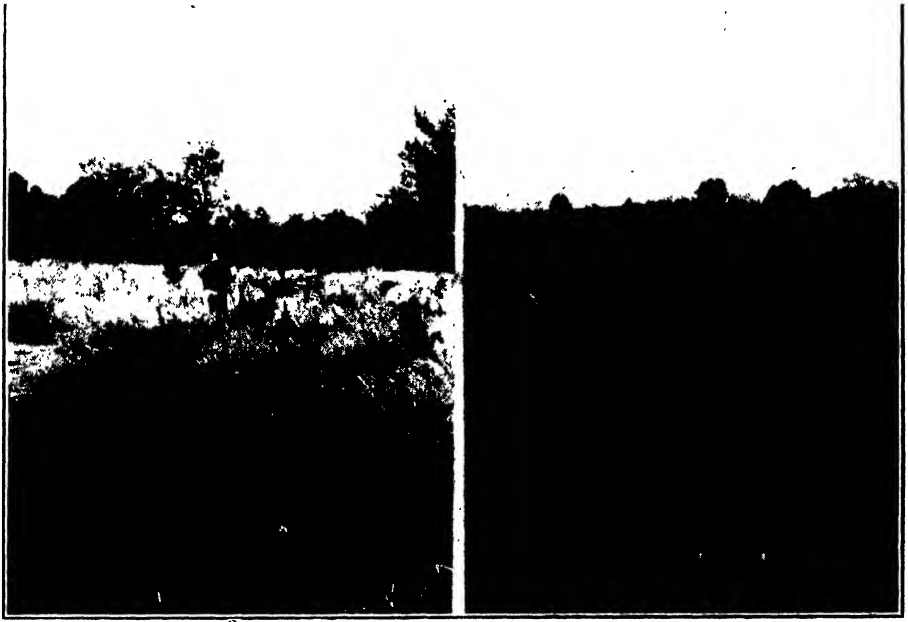
Of all the woody species the small shrubs exhibited the greatest mortality, whereas nearly all trees over 6 inches d.g.l. and most woody vines escaped destruction. Nearly all specimens of *Cerothamnus ceriferus, Rhus copallinum, Rubus* spp., and *Sambucus canadensis* observed had succumbed by the time of the June survey. On the other hand *Diospyros virginiana, Gleditsia triacanthos, Bignonia radicans,* and *Vitis* spp. exhibited no injury in June. In addition to the above species, no injured specimens of *Berchemia scan-*

[2] Plant nomenclature follows Small's *Manual of the Southeastern Flora.*

dens, Brunnichia cirrhosa, Celtis mississippiensis, Hicoria pecan, Ilex decidua, Platanus occidentalis, Quercus virginiana, Rufacer drummondii, or *Ulmus americana* were found in the autumn survey.

PIONEER VEGETATION

In our study of the revegetation of sediments, twelve plots at three widely separated stations were utilized. All these plots included ridges and swales in areas of considerable deposition as well as flats in an area of very slight alluviation. The vegetation was analyzed by phytosociological methods similar to those used by Penfound and Howard (1940) as modified from



2 3

FIG. 2. The feathery aspect of the Panicum associes is due to the witch grass, *Panicum capillare*. FIG. 3. In this example of the Cyperus-Panicum locies, *Panicum dichotomiflorum* is the predominant species.

Braun-Blanquet (1932). Each of the twelve plots was analyzed in June, August, and October of 1937. Similar analyses would have been made during the 1938 growing season had not the areas under investigation been drastically disturbed by clearing and by levelling of the dunes. Detailed phytosociological data are not presented in this paper owing to the exigence of space but will be made available to the reader upon request.

In general, it was found that the high and low ridges were occupied by a community which we have designated as the Panicum associes (fig. 2). Although the swales included several more hydric species than the ridges

we have designated the community found therein as the Cyperus-Panicum locies of the Panicum associes.

PANICUM ASSOCIES

In the spring survey the Panicum associes (on the ridges) was characterized by many species with a very low total coverage. The most important species at this time, in decreasing order of coverage comprised *Panicum capillare, Panicum dichotomiflorum, Digitaria sanguinalis, Cyperus esculentus, Mollugo verticillata, Eragrostis hypnoides,* and *Eragrostis pectinacea.* The first two of these species especially continued to increase in coverage throughout the season and are considered the community dominants. Of the total cover in the October survey over 70 per cent was contributed by *Panicum capillare* and about 10 per cent was occasioned by *P. dichotomiflorum.* The last four of the above species exhibited progressively lower coverage in the August and October surveys. This decline in importance as community components is probably due to their ephemeral habit. Calculation of total coverage, using the June survey as unity, gave the following relative values: June, 1; August, 1.7; October, 4.0.

The Panicum associes included a total of 102 species. Some of the species were encountered only in one or two of the surveys but the dominants were present throughout the growing season. As might be expected the number of species increased with the season (June, 73; August, 80; October, 85). Of the 102 species encountered 41.7 per cent were members of three families: Poaceae (18.4), Carduaceae (13.6), and Cyperaceae (9.6).

CYPERUS-PANICUM LOCIES

The alluvium in the swales varied from less than one foot to three feet in thickness and possessed a relatively high moisture content (20.4–52.4 per cent). Owing to the relatively high water content of the sediments, many hydric species developed which did not survive on the ridges. Despite the presence of these species it is felt that this community should be designated as a form of the Panicum associes, since 38 per cent of the total number of species and all of the dominant species were common to both communities (table 1).

TABLE 1

List of species encountered in plant communities developing on the deposits in the Bonnet Carré Floodway

A. Present in Panicum Associes only

Acalypha virginica	*Cerothamnus ceriferus*	*Diodia virginiana*
Ageratum conyzoides	*Chenopodium album*	*Dracopus amplexicaulis*
Ambrina ambrosioides	*Cirsium horridulum*	*Echinochloa crus-galli mitis*
Arundinaria tecta	*Croton capitatus*	*Eleusine indica*
Berchemia scandens	*Cynoctonum mitreola*	*Eragrostis spectabilis*
Bignonia radicans	*Cyperus* sp.	*Erigeron canadense*
Calyptocarpus vialis	*Digitaria sanguinalis*	*Fimbristylis diphylla*

Fraxinus americana
Heliotropium inundatum
Holcus halepensis
Ipomoea lacunosa
Iva ciliata
Leptilon canadense
Leptochloa uninerve
Maruta cotula
Medicago lupulina
Meibomia sp.
Mimosa strigillosa
Muhlenbergia schreberi
Paspalum conjugatum
Paspalum dilatatum
Paspalum urvillei
Persicaria hydropiper
Persicaria hydropiperoides
Persicaria sp.
Phyla nodiflora
Portulaca oleracea
Sambucus canadensis
Setaria lutescens
Sida carpinifolia
Sida rhombifolia
Sida spinosa
Solanum nigrum
Sporobolus cryptandrus
Strophostyles helvola
Svida asperifolia
Ulmus americana
Xanthoxalis micrantha
Xanthoxalis sp.

B. Common to Panicum associes and Cyperus-Panicum locies

Acnida tamariscina
Amaranthus deflexus
Ambrosia elatior
Ambrosia strifida
Ammannia coccinea
Ampelopsis arborea
Aster sp.
Bidens frondosa
Boehmeria cylindrica
Brunnichia cirrhosa
Chamaesyce humistrata
Cynodon dactylon
Cyperus erythrorhizos
Cyperus esculentus[b]
Cyperus filicinus microdontus
Cyperus inflexus
Cyperus virens Drummondii
Echinochloa colona
Equisetum robustum
Eragrostis hypnoides
Eragrostis pectinacea
Eupatorium capillifolium
Fimbristylis autumnalis
Fimbristylis vahlii
Fraxinus sp.
Ipomoea sp.
Leucospora multifida
Lythrum lanceolatum
Mikania scandens
Mollugo verticillata
Oenothera biennis
Panicum capillare[a]
Panicum dichotomiflorum[a, b]
Persicaria punctata
Phyla lanceolata
Platanus occidentalis
Pluchea petiolata
Polypremum procumbens
Populus balsamifera
Raimmania laciniata
Rynchospora corniculata
Salix longifolia
Salix nigra
Sesban Emerus
Spilanthes americana
Thyella tamnifolia
Verbena bonariensis
Verbesina alba
Xanthium sp.

C. Present in Cyperus-Panicum locies only

Celtis mississippiensis
Echinochloa Walteri
Ilysanthes inequalis
Juncus diffusissimus
Jussiaea decurrens
Jussiaea leptocarpa
Leptochola panicoides
Ludwigia glandulosa
Mimulus cylindrica
Paspalum repens
Phyllanthus carolinensis
Pontederia cordata
Rubus sp.
Rufacer Drummondii
Scirpus sp.
Toxicodendron sp.
Trachelospermum difforme
Typha latifolia

[a] Dominant in Panicum associes.
[b] Dominant in Cyperus-Panicum locies of Pancium associes.

The predominant species of the Cyperus-Panicum locies, in order of relative coverage in October, comprised *Panicum dichotomiflorum, Cyperus esculentus, Jussiaea leptocarpa, Typha latifolia, Salix* spp., and *Eragrostis hypnoides*. Of the above, the first two species contributed over 70 per cent of the total coverage in October and have been designated as the community dominants (fig. 3). As in the Panicum associes, there was a slight increase in the number of species as the season progressed (June, 49; August, 52; October, 63). A total of 67 species (table 1) was encountered in this community. Of this number, 37 per cent were members of three families (Poaceae, 13.7; Cyperaceae, 13.7; Carduaceae, 9.6).

COMPARISON OF SPECIES WITH PIONEERS OF OTHER SANDY DEPOSITS

The vegetation of the dune ridges in the floodway is not at all similar to that of the strand flora of the Gulf Coast. Of 21 species listed by Lowe (1921)

as occurring on sandy beaches in the state of Mississippi, only one was encountered in the floodway. In their study of Cat Island, Penfound and O'Neill (1934) include 54 herbaceous species in the three dune associations on Cat Island, Mississippi, but of these species only three were found on the floodway deposits. On the other hand, many plants developing on the floodway sediments have also been found on relatively barren areas on the river side of the artificial levees. Of the 43 species that Brown (1929) found in a bare area caused by the construction of a levee near Baton Rouge, 55 per cent were encountered in the Bonnet Carré floodway. On revegetating sandbars of the Mississippi River Davenport (1934) encountered a total of 62 species, of which 60 per cent were common to the floodway deposits.

The alluvium in the floodway was much finer than the strand deposits along the Gulf Coast but it is not known whether this difference is sufficient to account for the great diversity in vegetation. It should be pointed out, however, that the water content in coarse, sandy soils is often critically low. The pH of the floodway sediments was found to be relatively high (7.6 to 8.4) as compared with that of the strand deposits (6.0 to 7.2). A review of the literature suggests, however, that most species survive under pH differences as great as those between the alluvial and strand deposits. A further suggestion has been made that the weedy communities on the alluvial sediments are due primarily to water-borne seeds from upstream sources. Presumably the availability of migrules would explain the presence of these ruderal species. It is probable, however, that very few of these species could survive on the coarse sands of the strand and that water content and possibly pH may be important controlling factors in this habitat.

SUMMARY

1. The vegational changes which occurred in the Bonnet Carré Floodway, subsequent to its use in flood control during the late winter of 1937, are herein reported.

2. The diversion of flood waters through the floodway resulted in a striking depositional pattern of alternating ridges and swales, typical of "crevasse topography." The sediment consisted of fine sand with a small admixture of silt and clay.

3. The flood waters had no observable direct effect on the preëxisting vegetation but the sediments, especially where over one foot in depth, destroyed the herbaceous plants and much of the woody vegetation.

4. Vegetational studies, made in June, August, and October of 1937, revealed only one plant community and a wet phase of the same.

5. The Panicum associes, which develops on the ridges, was dominated by *Panicum capillare* and *Panicum dichotomiflorum* at the end of the growing season. The community included 102 species distributed largely in three families: Poaceae, Carduaceae, and Cyperaceae.

6. The Cyperus-Panicum locies, which develops in the swales, was dominated by *Cyperus esculentus* and *Panicum dichotomiflorum*. A total of 67 species was recorded, of which 37 per cent were members of the Poaceae, Carduaceae, and Cyperaceae.

7. The pioneer vegetation of the floodway deposits is very unlike that of the strand flora of the Gulf Coast but quite similar to that of the sandbars of the Mississippi River.

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[3] Investigation completed at Tulane University.

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69(4): 290–297 APRIL, 1942

THE GENUS CEPHALOCARPUS NEES (CYPERACEAE)

CHARLES L. GILLY

INTRODUCTION

Generic endemism in the tribe Lagenocarpeae is strikingly represented in the Sierra Pacaraima region of northern South America. The genus *Everardia* (4), the genus *Didymiandrum* (5), and the related genus *Cephalocarpus* have their center of distribution on this mountain complex, and the genus *Trilepis*—whose main distribution is in the similarly old mountainous region around Rio de Janeiro and in southern Minas Geraes—is represented by a single disjunct station in the Kanukus, an eastern outlier of the Pacaraima complex. These small genera are of interest because of the age of the area in which they are found, and because they exhibit certain characteristics which may be regarded as distinctly primitive within this family. Investigations which were begun while identifying Mr. G. H. H. Tate's Auyan-tepui[1] specimens of Cyperaceae (3) are continued in this paper.

The genus *Cephalocarpus*, established by Nees in the Flora Brasiliensis (7) with a single species *C. dracaenula*, has been merged with *Cryptangium* by Böckeler (1) and with *Lagenocarpus* by Pfeiffer (9); neither of these treatments, however, is acceptable on the basis of a study of the more recently collected material. Clarke (2) accepted Böckeler's merger, but other botanists who have worked with the Cyperaceae have usually recognized *Cephalocarpus* as a distinct genus.

In 1921 (8), when placing *Cryptangium* in synonymy under *Lagenocarpus*, Pfeiffer removed Böckeler's *Cryptangium dracaenula* (based on *Cephalocarpus dracaenula* Nees) from the enlarged and emended genus as "*Cephaloscirpus* [sic!] *Dracaenula* Nees," thereby recognizing *Cephalocarpus* as distinct. Then, in 1922 (9), he reduced the genus to subgeneric status under *Lagenocarpus;* in this new subgenus he placed *L. polyphyllus* Kuntze, *L. clarkei* H. Pfeiff. (nom. nov. for *Cryptangium strictum* C. B. Clarke), *L. humilis* Kuntze, and *L. comatus* H. Pfeiff. The only apparent basis for such inclusion seems to be the anomalous and dissimilar habits of these four species which are as unlike each other as they are unlike *C. dracaenula* in appearance. Inflorescence, spikelet, and achene characters of these species certainly do not indicate such close relationship as Pfeiffer would infer. On page 72 of the same paper, "*for the convenience of those*

[1] "Tepui," an Indian dialect name, means "mountain"; thus, Auyan-tepui is, in reality, Mt. Auyan. Mt. Roraima is known by the natives of the region as Roraima-tepui, and Mt. Duida as Duida-tepui.

who are inclined to accept smaller genera,"[2] he makes the combinations *Cephalocarpus comatus*, *C. humilis*, *C. polyphyllus*, and *C. clarkei*. The last of these needlessly created combinations was, incidentally, improperly made, for it was based on *Cryptangium strictum* C. B. Clarke; while the specific epithet *strictus* had already been used in the genus *Lagenocarpus*, thus necessitating a new name for the species in that genus, it had not been used in *Cephalocarpus*. In 1924 (10) Pfeiffer added another anomalous species, *L.* (*Cephalocarpus*) *schenckianus* H. Pfeiff., to the subgenus *Cephalocarpus*.

The five species transferred to his new subgenus by Pfeiffer are untenable in *Cephalocarpus* Nees (see list of excluded species at end of this paper); whether they may be retained in *Lagenocarpus*, or whether they must be referred to the genera under which they were originally described, is not a matter for consideration in the present paper.

Cephalocarpus, then, has remained as a monotypic genus for almost one hundred years. While studying the Tate collections of Cyperaceae from Mt. Roraima, Mt. Duida, and Auyan-tepui, I discovered that certain specimens, provisionally referred to the genus "*Everhardia*" by Dr. Britton in the report on the flora of Mt. Duida (6), represented undescribed species of *Cephalocarpus*. At the time I published *C. rigidus* and *C. longibracteatus* in the Auyan-tepui report (3), I had not examined the type of *C. dracaenula* Nees; hence, on page 153 of that paper, I stated, "It is of interest to note that *C. Dracaenula* has been collected on both Mt. Roraima (*Tate 285*), and Mt. Duida (*Tate 800*)." This statement was made because I had provisionally associated these two specimens with Nees' original description and plate (7). My recent discovery—in the Gray Herbarium—of a specimen which is undoubtedly an isotype of the Nees species, has shown without question that *Tate 285* and *800* cannot be referred to *C. dracaenula*. Further study indicates that *Tate 720* and *721*, which I referred (3) to *C. rigidus*, can scarcely be regarded as conspecific with *Tate 1346* and must therefore be considered as a distinct entity. Another specimen, *Tate 1035*, in the herbarium of the New York Botanical Garden, proves also to be specifically distinct.

Cephalocarpus should be referred to the tribe Lagenocarpeae,[3] and appears to be most nearly related to the genus *Evarardia*. From this genus, which has elongated and compound inflorescences, achenes which taper into conical beaks, and 6 stamens subtended by each staminiferous glume, *Cephalocarpus* differs in having capitate-condensed inflorescences, achenes with clavate beaks and 2–3 stamens subtended by each staminiferous glume.

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[2] ". . . ut iis, qui parva genera anteponunt, genus solutum Cephalocarpus discernendae sint."

[3] This tribe is usually called the Cryptangieae, but because of the doubtful status of the genus *Cryptangium* I am following Pfeiffer in the choice of a tribal name.

From *Lagenocarpus,* which has terminal inflorescences and lacks a perianth, *Cephalocarpus* differs in having axillary inflorescences and a definite perianth.

With one exception, the distribution of *Cephalocarpus* parallels that of *Everardia* (4), both genera having been collected from Auyan-tepui, Mt. Duida, and Mt. Roraima of the Sierra Pacaraima complex. In addition to these localities *Cephalocarpus* has been found in the Cupati Mountains (the generic type locality), a low range of foot-hills geologically similar to the Pacaraimas, near the Caqueta (= the upper Japuru) River in what is now eastern Colombia.

I wish to thank Dr. J. H. Barnhart for bibliographical assistance, and Dr. C. A. Weatherby for the loan of a specimen and type photographs which are deposited in the Gray Herbarium.

TAXONOMIC TREATMENT[4]

CEPHALOCARPUS Nees, in Mart. Fl. Bras. **2** (1): 162, *t. 18.* 1842.

Lagenocarpus, subgenus *Cephalocarpus* H. Pfeiff. Repert. Spec. Nov. **18**: 91. 1922.

Terrestrial or epiphytic perennials. Stems rhizomatous, woody, simple or branched, surrounded by the persistent and more-or-less ragged sheaths of previous years' leaves and partially supported by prop-roots. Leaves linear, acute, somewhat flattened below, bicarinate above, crowded at apices of stem or its branches, 3-ranked but sometimes so closely crowded as to appear many-ranked. Flowering culms axillary, solitary and basally enclosed by a sheath, or 3–8 together and all basally enclosed by a single sheath; basal sheaths (prophyllons) membranous, 2-keeled, bifid by the prolongation of the keels beyond the tubular portion. Inflorescences of simple or compound capitate clusters of monoecious spikelets subtended by 2–several leaf-like bracts, the inflorescences usually containing both kinds of spikelets or some of them composed wholly of staminate spikelets. Spikelets sessile or short-pediceled, the pedicels basally bracteolate by 1–4 minute, hyaline, nerveless, acute or bifid scales. Staminate spikelets 2–5-flowered; empty outer glumes 2–5, staminiferous inner glumes 2–7, sometimes 1 or 2 of the innermost empty; stamens subtended by each staminiferous glume 2 (or rarely 1 or 3); filaments flattened, exserted, persistent; anthers linear, basifixed, 2-celled, the connective prolonged between the thecae into a minute pubescent mucro. Pistillate spikelets usually fewer in number than the staminate, 1-flowered, the ovary surrounded by 4–6 spirally imbricated glumes; rarely (and abnormally) the second or third glume subtends a single staminate flower. Achenes terete to obtusely triangular, obovoid, more or less conspicuously marked by 3 longitudinal tricarpellary lines, the apex depressed and centrally apiculate, the apiculation covered by a clavate, pubescent or glabrous, persistent or tardily deciduous beak formed by a prolongation of the outer pericarp layer. Style short, deciduous; stigmas 3, terete, linear, elongated, exserted; both style and stigmas densely and mi-

[4] Specimens examined in the preparation of this paper are deposited in the herbarium of the New York Botanical Garden (NY), and in the Gray Herbarium (GH).

nutely glandular-pubescent. Perianth cupular, usually persistent on the base of the achene, formed by partial marginal fusion of 3 minute ciliate-margined hypogynous scales.

TYPE SPECIES: *Cephalocarpus dracaenula* Nees.

KEY TO THE SPECIES

Flowering culms 3–8 together in the axil of a single leaf, all basally enclosed within a single tubular sheath; beak of achene tardily deciduous, exposing the terminal apiculation of the achene body (Subgenus 1. *Eucephalocarpus*[5]).

Beak of achene glabrous, shining, yellow, the body bright reddish-brown; perianth scales orbicular; leaves thinly white-pilose on the margins . . . . . . 1. *C. confertus*

Beak of achene pubescent, both beak and body dull, stramineous; perianth scales acutely triangular; leaves coarsely ciliate at base, the margins above minutely scabrous . . . . . . 2. *C. dracaenula*

Flowering culms solitary in the leaf axils, each enclosed by a basal tubular sheath; beak of achene persistent, permanently attached to and covering the terminal apiculation of the achene body (Subgenus 2. *Neocephalocarpus*[6]).

Leaves flexible, less than 1.5 mm. in width, 8–15 cm. long; achenes 0.75 mm. in diam. . . . . . . 3. *C. lineariifolius*

Leaves rigid, 1.5 mm. or more in width, less than 8 cm. long; achenes 1–1.5 mm. in diam.

Inflorescences simply-captitate; lowest bract subtending the inflorescence less than 10 mm. long, its sheath glabrous or finely appressed-pubescent, the margins of the free portion appressed-pubescent or scabrid.

Outer glumes of spikelets bifid at apices, their midribs prolonged between the teeth into prominent scabrid mucros; leaf margins scabrid . . . . . . 4a. *C. rigidus* var. *typicus*

Outer glumes of spikelets tapering gradually into the acute or mucronate apices, never bifid; leaf margins and midrib beneath appressed-pubescent . . . . . . 4b. *C. rigidus* var. *mucronatus*

Inflorescences compound-capitate; lowest bract subtending the inflorescence 10–25 mm. long, its sheath densely white-villous, the margins of the free portion pilose . . . . . . 5. *C. longibracteatus*

SUBGENUS 1. EUCEPHALOCARPUS GILLY.

1. **Cephalocarpus confertus** Gilly, sp. nov.

Rhizomata crassa ramosa, ad 5 cm. alta: folia valde conferta, late linearia acuta bicarinata, 3–7 cm. longa, 2–3.5 mm. lata, glabra margine et costa albo-villosa exceptis; culmi floriferi ad 1.5 cm. longi, 3–5 aggregati in axillibus foliorum; bracteae inflorescentiam subtendentes 2–4, inferiores 4–7 mm. longae; spiculae masculae 4–6 mm. longae, 0.75–1 mm. diam., glumis vacuis lanceolatis acutis glabris, glumis fertilibus oblongis acutis hyalinis; spiculae foemineae masculas aequantes, glumis 4–5, lanceolatis acutis vel mucronatis; achaenia subteretia obovoidea glabra rubro-brunnea, 1.25–1.5 mm. longa,

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[5] **Eucephalocarpus** Gilly, subgen. nov. Culmi floriferi 3–8 aggregati axillibus foliorum; rostro subpersistente, deciduus ad ultimum apiculus achaenium expositio. Subgeneris typus: *Cephalocarpus dracaenula* Nees.

[6] **Neocephalocarpus** Gilly, subgen. nov. Culmi floriferi solitarii axillibus foliorum; rostro persistente; apiculus achaenium obscurus. Subgeneris typus: *Cephalocarpys rigidus* Gilly.

0.75 mm. diam., rostro clavato glabro luteo subpersistente, 0.75–1 mm. longo, apiculo 0.7 mm. longo; squamellae hypogynae orbiculares, pilis paucis squamellas aequantibus.

In this species the second or third glume of the pistillate spikelet rarely (and apparently abnormally) subtends a single staminate flower.

TYPE: VENEZUELA—TERRITORIO AMAZONAS: Mount Duida, dry ridge tops, Savanna Hills, Alt. 4400 ft., Aug. 1928–Apr. 1929, *Tate 800* (NY). Also examined: VENEZUELA—BOLIVAR: Mount Roraima, on the great sand-stone boulders, Philipp Camp, alt. 5200–6000 ft., Nov. 7, 1927, *Tate 285* (NY).

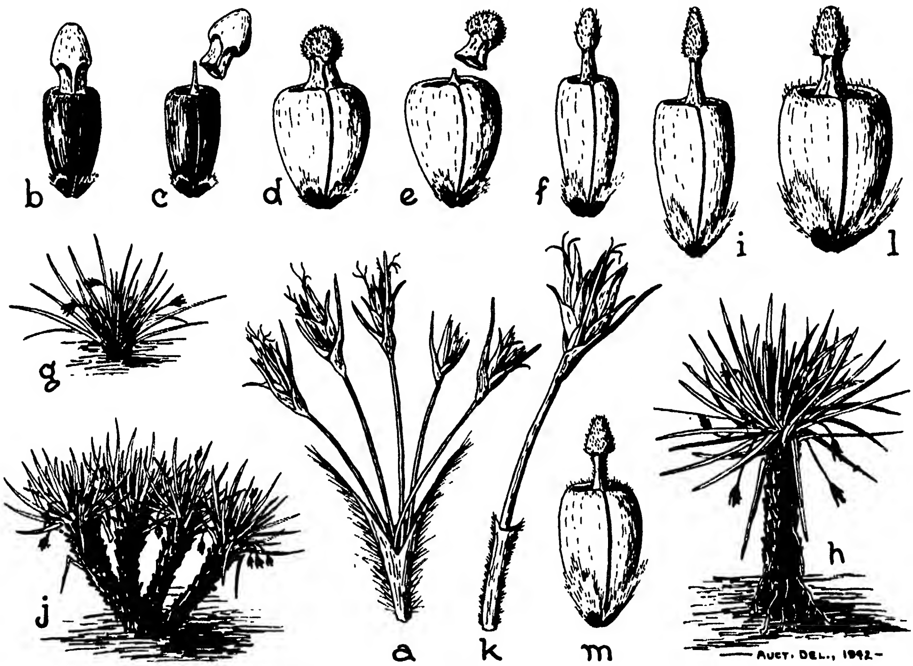

FIG. 1. *Cephalocarpus:* habit sketches ×0.5; flowering culms ×2.5; achenes ×5. *C. confertus* Gilly (drawn from *Tate 800*): *a*, aggregated flowering culms surrounded by a single basal sheath, characteristic of the subgenus *Eucephalocarpus; b*, achene, beak attached; *c*, achene, beak detached. *C. dracaenula* Nees (drawn from *Martius s.n.*): *d*, achene, beak attached; *e*, achene, beak detached. *C. lineariifolius* Gilly (drawn from *Tate 1035*): *f*, achene. *C. rigidus* var. *typicus* Gilly (drawn from *Tate 1346*): *g*, young plant, the rhizome not yet evident above ground; *h*, old plant, showing well developed rhizome; *i*, achene. *C. rigidus* var. *mucronatus* Gilly (drawn from *Tate 720*): *j*, old plant, showing branched aerial rhizome; *k*, solitary flowering culm enclosed by basal sheath, characteristic of the subgenus *Neocephalocarpus; l*, achene. *C. longibracteatus* Gilly (drawn from *Tate 1345*): *m*, achene.

2. CEPHALOCARPUS DRACAENULA Nees, in Mart. Fl. Bras. **2** (1): 162. *t. 18.* 1842. *Cryptangium dracaenula* Böckl. Linnaea **38**: 415. 1874. *Lagenocarpus dracaenula* H. Pfeiff. Repert. Spec. Nov. **18**: 92. 1922.

Rhizome slender, to at least 30 cm. tall, simple or branched. Leaves loosely clustered, rigid and more or less erect, 2–3 mm. wide, 7–8 cm. long, coarsely ciliate at mouth of sheaths, otherwise glabrous except for scabrous margins

and under surface of midrib. Flowering culms 4–8 together in the axil of a single leaf, flattened with scabridulous margins; basal sheath 1.5 cm. long, deeply bifid at the apex. Bracts subtending the inflorescence 2–3, glabrous except for the coarsely ciliate margins of the free portions, the lowermost 5–6 mm. long. Staminate spikelets 5 mm. long, about 1 mm. in diam.; sterile glumes 2–4, broadly lanceolate, abruptly tapering into a prominent mucro; fertile glumes 3–5, lanceolate, acute, the midrib obsolete. Pistillate spikelets 5 mm. long, 1–1.5 mm. in diam., glumes 5–6, narrowly ovate to lanceolate, glabrous, mucronate, mucronulate, or acute, the mucro of the outermost sometimes coarsely ciliate. Achene stramineous, the body 1.5 mm. long, 1.25 mm. in diam., obovoid, subterete, glabrous except for the sparse pubescence at the apex. Beak about 0.75 mm. long, the apical knob hemispherical, pubescent; the beak tardily deciduous, thus exposing the 0.2–0.3 mm. apiculation in the center of the depressed apex of the achene body. Style 2–3 mm. long; stigmas 3 mm. or less in length, dark red-brown. Hypogynous scales triangular, acute, the marginal hairs sparse, about 0.3 mm. long.

Specimen examined: COLOMBIA—CAQUETA: [Cupati Mts.] cataracts of the Rio Japuru (=Rio Caqueta), 1841, *Martius s.n.* (GH). This specimen is an isotype.

SUBGENUS 2. NEOCEPHALOCARPUS GILLY.

3. **Cephalocarpus leariifolius** Gilly, sp. nov.

Rhizomata gracilia simplicia vel ramosa, ad 15 cm. alta; folia linearia acuta bicarinata glabra margine et costa appresso-pubescentibus exceptis, 8–15 cm. longa, 1.5 mm. lata minusve; culmi floriferi solitarii in axillibus foliorum, 6–8 cm. alti; bracteae inflorescentiam subtendentes 10 mm. longae; spiculae masculae 3.5–5 mm. longae, glumis sterilibus 2–3, lanceolatis acutis; antherae 1–1.25 mm. longae; spiculae foemineae 4–5 mm. longae, glumis 4–5, lanceolatis acutis vel mucronatis marginibus et mucrone pubescentibus; achaenia subteretia obovoidea glabra straminea, 1.75–2 mm. longa, 0.75 mm. diam., rostro stramineo persistente glabrato vel vix pubescente, 0.75–1 mm. longo; squamellae hypogynae suborbiculares, ciliis subcopiosis quam corpore achaenii 3–plo brevioribus.

TYPE: VENEZUELA—TERRITORIO AMAZONAS: Mount Duida, flat near stream at Central Camp, epiphytic on bark of trees, alt. 4800 ft., Dec. 20–28, 1928, *Tate 1035* (NY).

4. CEPHALOCARPUS RIGIDUS Gilly apud Gleason & Killip, Brittonia **3**: 152. 1939.

In the report on the flora of Auyan-tepui (3), I referred two specimens from Mt. Duida to *C. rigidus;* further study of these specimens indicates that they can scarcely be regarded as conspecific with *Tate 1346* (the type of *C. rigidus*) from Auyan-tepui, and it therefore becomes necessary to describe them as a separate entity. However, from the specimens available I do not feel justified in recognizing the Duida material as a distinct species and so—for the present—it seems better to regard *C. rigidus* as a collective species composed of two geographically isolated varieties. Since the illustrations of the glumes (l.c., *fig. 2; a, b*) do not represent the typical form of the species, and since the original description of *C. rigidus* combines the characters of both varieties, it seems necessary to here describe and differentiate these varieties.

4a. CEPHALOCARPUS RIGIDUS Gilly var. **typicus** Gilly, var. nov. *Cephalocarpus rigidus* Gilly apud Gleason & Killip, Brittonia **3**: 152. *fig. 2, c.* (as to type, but not as to description). 1939.

Rhizomata simplicia, ad 6 cm. alta; folia valde conferta rigida coriacea glabra margine scabra excepta, ad 2 mm. lata, 3–4 cm. longa; culmi floriferi solitarii in axillibus foliorum, 2–3 cm. alti; spiculae masculae ad 4 mm. longae, glumis sterilibus 3–5 ovatis vel lanceolatis bifidis aristatis vel mucronatis, glumis fertilibus 3–4 oblongis vel lanceolatis acutis hyalinis; spiculae foemineae 4.5–6 mm. longae, glumis 5–6 lanceolatis bifidis hyalinis aristatis vel mucronatis, apicibus pubescentibus; achaenia subteretia obovoidea glabra vel ad apicem pubescentia, 1.75–2 mm. longa, ad 1 mm. diam., rostro pubescente persistente stramineo, 1.25 mm. longo; squamellae hypogynae orbiculares, ciliis copiosis quam corpore achaenii 3- vel 2-plo brevioribus.

TYPE: VENEZUELA—BOLIVAR: Auyan-tepui, 2200 m., Dec. 1937, *Tate 1346* (NY).

4b. CEPHALOCARPUS RIGIDUS Gilly var. **mucronatus** Gilly, var. nov. *C. rigidus* Gilly apud Gleason & Killip, Brittonia **3**: 152. *fig. 2, a, b.* (in part, but not as to type). 1939.

Rhizomata simplicia vel ramosa, ad 6 cm. alta; folia 2–8 cm. longa, 2–2.5 mm. lata, glabra marginibus et costa appresso-pubescentibus exceptis; culmi floriferi solitarii in axillibus foliorum, 2–3.5 cm. alti, ad basim pilosi; bracteae inflorescentiam subtendentes 5–9 mm. longae; spiculae masculae 5–6 mm. longae, glumis vacuis 3–5, lanceolatis mucronatis, glumis fertilibus 3–4, lanceolatis hyalinis mucronulatis vel acutis; antherae 3 mm. longae; spiculae foemineae ad 5 mm. longae, glumis 4–6, ovatis vel lanceolatis aristatis; achaenia teretia obovoidea, 2 mm. longa, ad 1.5 mm. diam.; rostro 1–1.25 mm. longo persistente pubescente; squamellae hypogynae suborbiculares, ciliis copiosis quam corpore achaenii 3-plo brevioribus.

TYPE: VENEZUELA—TERRITORIO AMAZONAS: Mt. Duida, on soil, Ridge 16, 6800 ft., 1928–1929, *Tate 721* (NY). Also examined: from the same locality, a dwarfed form growing on rock, *Tate 720* (NY).

5. CEPHALOCARPUS LONGIBRACTEATUS Gilly apud Gleason & Killip, Brittonia **3**: 153. *fig. 2, d–f.* 1939.

Rhizome short, simple or branched. Leaves loosely clustered, 2–3.5 mm. wide, 4–8 cm. long, pale green, firm but not coriaceous, sparsely to densely pubescent. Flowering culms solitary in leaf-axils, flattened, 0.5–1 mm. wide, densely pilose at base, glabrate above, exserted 4–7 cm. from the sheath of the subtending leaf; basal sheath 1–1.5 cm. long, minutely and bluntly bifid at the apex. Inflorescence compound, consisting of several pedicellate spikelets and several pedicellate spikelet clusters, the whole subtended by 2–3 leaf-like bracts; lowermost bract 10–25 mm. long, others smaller, sheaths of all densely white-villous, margins of the free portions long-pilose; bracts subtending the individual spikelet clusters similar but smaller. Staminate spikelets 4.5–6 mm. long, 0.75–1 mm. diam.; sterile glumes 3–4, broadly lanceolate, hyaline except for the prominent midrib, acute to mucronate; fertile glumes 2–3, oblong to narrowly lanceolate, acute, hyaline. Pistillate spikelets 4–5 mm. long, 1–1.5 mm. in diam.; glumes 5–6, the outer broadly

lanceolate, abruptly aristate, the inner lanceolate, acute. Achene straw-colored, the body 1.6–1.8 mm. long, 1–1.3 mm. in diam., minutely pubescent at the apex; beak persistent, about 1 mm. long, densely apically pubescent. Hypogynous perianth scales orbicular; marginal cilia copious, one-third to one-half as long as the body of the achene.

TYPE: VENEZUELA—BOLIVAR: Auyan-tepui, 2200 m., Dec. 1937, *Tate 1345* (NY).

SPECIES EXCLUDED FROM THE GENUS

1. *Cephalocarpus clarkei* H. Pfeiff. Repert. Spec. Nov. **18**: 72. 1922. = *Cryptangium strictum* C. B. Clarke, Kew Bull. Add. Ser. **8**: 66. 1908. (*Lagenocarpus clarkei* H. Pfeiff. Repert. Spec. Nov. **18**: 92. 1922. Not *Lagenocarpus strictus* Kuntze, Rev. Gen. 754. 1891).

2. *Cephalocarpus comatus* (Böckl.) H. Pfeiff. Repert. Spec. Nov. **18**: 72. 1922. = *Cryptangium comatum* Böckl. Flora **65**: 351. 1882. (*Lagenocarpus comatus* H. Pfeiff. Ber. Deuts. Bot. Ges. **39**: 131. 1921).

3. *Cephalocarpus humilis* (Nees) H. Pfeiff. Repert. Spec. Nov. **18**: 72. 1922. = *Acrocarpus humilis* Nees, in Mart. Fl. Bras. **2** (1): 161. 1842. (*Lagenocarpus humilis* Kuntze, Rev. Gen. 754. 1891).

4. *Cephalocarpus polyphyllus* (Nees) H. Pfeiff. Repert. Spec. Nov. **18**: 72. 1922. = *Acrocarpus polyphyllus* Nees, in Mart. Fl. Bras. **2** (1): 160. 1842. (*Lagenocarpus polyphyllus* Kuntze, Rev. Gen. 754. 1891).

5. *Cephalocarpus schenckianus* (Böckl.) H. Pfeiff. Repert. Spec. Nov. **20**: 44. 1924. = *Cryptangium schenckianum* Böckl. Cyp. Nov. **2**: 27. 1890. (*Lagenocarpus schenckianus* H. Pfeiff. Ber. Deuts. Bot. Ges. **39**: 132. 1921).

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NEW YORK, NEW YORK.

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10. ————. Additamenta ad cognitionem generis *Lagenocarpus*, IV. Repert. Spec. Nov. **20**: 42–45. 1924.

BULLETIN OF THE TORREY BOTANICAL CLUB

69(4): 298–316 APRIL, 1942

NORTH AMERICAN RANUNCULI—IV

LYMAN BENSON

The first and second articles in this series of five (Bull. Torrey Club **68** (3, 7): 157–172, 477–490. 1941) deal with the subgenus *Euranunculus*, section *Chrysanthe*, and the third (Bull. Torrey Club **68**: 640–659. 1941) is a treatment of the sections *Echinella* and *Epirotes*. In this paper the sections *Flammula* and *Hecatonia* are discussed, concluding the subgenus *Euranunculus*. The present delimitation of subgenera and sections was published in an article entitled "The North American subdivisions of *Ranunculus*" (Am. Jour. Bot. **27** (9): 799–807. 1940). Detailed specific descriptions are reserved of the North American Flora.

SECT. 4. FLAMMULA (WEBB) L. BENSON

Perennials; achenes 1.2–3.2 mm. long, the beaks 0.3–1.5 mm. long or in typical *R. Flammula* 0.1–0.2 mm. long; petals 5–10, conspicuous and usually large, longer than the sepals, except in *R. oresterus*.

- Nectary scale ciliate along the distal margin, the adjacent surface of the petal with some hairs; achenes about 3–3.2 mm. long; receptacle short-pubescent; achenes pubescent, each with a flat, winged stalk; stems scapose . . . . . . 48. *R. Collomae*.
- Nectary scale glabrous, the petal also glabrous; achenes 1.2–2.5 mm. long; receptacle glabrous except sometimes in *Ranunculus Populago*; achenes glabrous except in *Ranunculus alismaefolius* var. *Lemmonii*, never stalked.
  - Roots not with light-colored, fusiform, thickened storage regions at the bases.
    - Stems never rooting.
      - Roots glabrous in their mature portions; petals 6–16 mm. long, 3–8 mm. broad, exceeding the speals, obovate or in var. *montanus*, which has almost always 10 petals, oblanceolate or narrowly oblong and 2–3 mm. broad; basal leaf not broadest at the middle and tapering to the acute ends . . . . . . 49. *R. alismaefolius*.
      - Roots densely and finely canescent their whole length; petals not exceeding the sepals, narrowly oblong, 3–5 mm. long, 1–2 mm. broad, always 5; basal leaf broadest at the middle and tapering to the acute ends . . . . . . 50. *R. oresterus*.
    - Stems rooting adventitiously at at least the lower nodes (rarely not rooting in typical *R. Flammula*, which is restricted to Newfoundland and Nova Scotia).
      - Achene body about 2 mm. long, nearly rectangular, the beak 1.1–1.3 mm. long; stem 5–10 dm. long, 5–8 mm. in diameter at the base, rooting at only the lower nodes, the distal portion ascending . . . . . . 51. *R. ambigens*.
      - Achene body 1.3–1.7 mm. long, obovate, the beak 0.1–0.8 mm. long.
        - Cauline leaves lanceolate, oblanceolate, or linear . . . . . . 52. *R. Flammula*.

Cauline leaves ovate, ovate-lanceolate, or rarely subcordate.
Cauline petioles sheathing the stem; stem filiform, never fistulous .... 52C. *R. Flammula* var.
Cauline petioles not sheathing the stem; stem often fistulous .... 53. *R. hydrocharoides.*
Roots each with a small, light-colored, fusiform-thickened storage region at the base, this structure 1–2 mm. in diameter.
Stems rooting adventitiously at the nodes; leaves alternate, the flowers never in cymes .... 54. *R. Gormanii.*
Stems never rooting; leaves commonly opposite, the flowers usually in cymes .... 55. *R. Populago.*
Annuals; achenes 0.6–1 or rarely 1.5 mm. long, the beaks 0.1–0.2 mm. long.
Petals 5–9, large and conspicuous, about twice as long as the sepals; styles in anthesis 0.5 mm. long, filiform, deciduous in fruit; head of achenes hemispherical or ovoid .... 56. *R. laxicaulis.*
Petals 1–3, minute and inconspicuous, shorter than or equal to the sepals; styles in anthesis 0.1–0.2 mm. long.
Sepals 5; upper cauline leaves linear to lanceolate or oblanceolate or very narrowly elliptic, sessile; achenes oblong-obovate, 0.6–1 mm. long, smooth, reticulate, or papillate, the units of the reticulate pattern perhaps 0.03 mm. in diameter when visible .... 57. *R. pusillus.*
Sepals 3; upper cauline leaves ovate, petioled; achenes obovate, about 1.5 mm. long, reticulate, the units of the pattern about 0.1 mm. in diameter .... 58. *R. alveolatus.*

48. **Ranunculus Collomae** L. Benson, sp. nov. Glabrous terrestrial perennials; roots 6–10, remarkably stout, 2–3 mm. in diameter; stems scapose, prostrate or ascending, not rooting adventitiously, 3–5 cm. long up to the lowest pedicel, 1.5–2.5 mm. in diameter, 1–3-flowered; radical leaf blades simple, oblanceolate to obovate, 2.5–5 cm. long, 0.8–1.5 or 2 cm. broad, entire, proximally attenuate and distally acute; petioles 2.5–3.5 cm. long; stipular leaf bases 1–1.5 cm. long, cauline leaves 1–3, opposite or alternate, lanceolate to oblanceolate, sessile; pedicels 2–5.5 cm. long in flower and 6–10 cm. long in fruit, glabrous; sepals 5, yellowish-green tinged with purple, spreading, elliptic, 5 mm. long, 3 mm. broad, pilose dorsally, about half the length of the petals, promptly deciduous; petals 5, yellow, obovate, 7–12 mm. long, 5–7 mm. broad, the nectary scale forming a pocket, broadly oblong, ciliate on the distal margin; stamens about 40–50; achenes 50–100 in an ovoid head 10–12 mm. long, 9–10 mm. in diameter, each achene obovoid, 3–3.2 mm. long, 2–2.2 mm. dorsoventrally, 1–1.2 mm. laterally, smooth, finely pubescent, the margin conspicuous, the achene beak slender, 1 mm. long, apically curved or recurved; receptacle ovoid-cylindrical, 1–2 mm. long in flower, 8–9.5 mm. long in fruit, finely pubescent.

Herba terrestris perennis glabra; radicibus 2–3 mm. diametro; caulibus non e nodis radicantibus, scaposis, 1 dm. longis, 1.5–2.5 mm. diametro, 1–3-floris; foliis oblanceolatis vel obovatis, integris, 2.5–5 cm. longis, 0.8–1.5 rariter 2 cm. latis; petiolis 2.5–3.5 cm. longis; petalis 5, flavis, obovatis, 7–12 mm. longis, 5–7 mm. latis; nectarii squamulis ciliatis, oblongis, latere non liberis; carpellorum capitulo ovoide, 10–12 mm. longo, 9–10 mm. diametro; acheniis 40–50, obovoidis, canescentibus, 3–3.2 mm. longis, rostro 1 mm. longo, curvato vel recurvato; receptaculo ovoide, 8–9.5 mm. longo, canescente.

Open, dry ground under yellow pines at 2,300–2,500 meters elevation; La Sal Mountains, southeastern Utah to Coconino County, Arizona. Western pine forest. April.

Type collection: near Bugglen (Buglan) Hill, South Rim of the Grand Canyon, Coconino County, Arizona: coll. April 10, 1941, *Mrs. Rose E. Collom 999.* TYPE in the Herbarium of the University of Arizona. ISOTYPE, B.

Significant specimens: UTAH: 20 miles west of Blanding, La Sal Mountains, *Harrison & Williams 5984, UA,* Utah[1]; ARIZONA: Jacobs Lake, Kaibab Forest, *Maguire 13550,* UA, Utah; South rim of Grand Canyon at Bugglen (Buglan) Hill, *Collom BHI,* May 8, 1940, *US, 999,* April 10, 1941, *UA, B;* 1½ miles north of Foxburrow (Foxborough) Ranch, on Schnably Hill Road, Oak Creek Canyon, *Wetherill 1,* April 7, 1940, *UA, MNA*[1], *B.*

This species has affinity to *Ranunculus glaberrimus* Hook. var. *ellipticus* Greene, and in some respects it is a connecting link between the sections *Epirotes* and *Flammula* although it is definitely in the latter section. The following characters show relationship to var. *ellipticus:* (1) presence of only 6–10 large, coarse roots, (2) habitat in dry soil, (3) pubescent achenes, (4) winged stipe of the achene, (5) ciliate nectary scale (ciliate or glabrous in *R. glaberrimus*). Only four species of *Ranunculus* in North America have ciliate nectary scales. They are *R. cardio phyllus, R. arizonicus,* and *R. glaberrimus* in the section *Epirotes* and *R. Collomae.* The new species, *R. Collomae,* is most closely related to *R. alismaefolius* Geyer.

49. RANUNCULUS ALISMAEFOLIUS Geyer. ex Benth. Pl. Hartw. 295. 1848. *R. Bolanderi* Greene, Bull. Calif. Acad. **2**: 58. 1886. *R. Flammula alismifolius* Clements & Clements, Rocky Mt. Flowers 6. 1914, *nomen nudum.*

Glabrous terrestrial or almost palustrine perennials; roots usually 15–30, slender, about 1.5–2 mm. in diameter; stems 3–8 dm. long, mostly 4–6 mm. in diameter, rather freely-branching above, fistulous; radical leaf blades lanceolate, 4–12 cm. long, 1–3 cm. broad, entire or often serrulate, thick, proximally tapering into the petiole and distally blunt; petals 5, obovate, about 10 mm. long, about 5 mm. broad, the nectary scale 1 mm. long, forming a pocket 0.5 mm. deep, the distal half free; achenes 30–50 in a hemispherical to subglobose head 5–7 mm. long and 6–8 mm. in diameter, each achene cuneate-obovate, 2.5 mm. long, 1.7 mm. dorsoventrally, 1 mm. laterally, smooth, glabrous, the margin inconspicuous, the achene beak slender but thick at the base, 0.7–0.9 mm. long, not recurved; receptacle pyriform, 1–2 mm. long in flower and 4–5 mm. long in fruit, glabrous.

Muddy lake borders and ditches at low elevations in the coastal region and up to 1,000 or 1,300 meters inland; Vancouver Island to Eastern British Columbia and Northern Idaho; Lewis County, Falcon Valley, and the eastern border of Washington; Wallowa Mountains, Eastern Oregon, and from the Willamette Valley to the Seaward North Coast Ranges as far south as Mendocino County, California. Northwestern coniferous forest and Palouse grassland. May and June.

[1] Symbols not used previously. *Utah,* Intermountain Herbarium of Utah State College; *MNA,* Museum of Northern Arizona, Flagstaff.

Type collections: (1) *R. alismaefolius*, ". . . Geyerianis e montibus scopulosis. . . " "High grassy plains of Couer d'Aleine. (n. 306), Idaho, cf. Hook. London Jour. Bot. **6**: 66. 1847. (2) *R. Bolanderi*, "Long Valley, Mendocino County California, May, 1886, H. N. Bolander, No. 4730." The TYPE is *HGr. 2409*. (3) *R. flammula alismifolius, nomen nudum*, referred here because of the similarity of the names *alismifolius* and *alismaefolius*, although the authors must have had in mind *R. alismaefolius* var. *montanus*, since it is the only variety occurring in the range covered in the book.

KEY TO THE VARIETIES

Petals 5, obovate.
- Receptacle elongated by 3–5 mm. in fruit, pyriform; stems 3–8 dm. long; leaves lanceolate, often serrulate. .... 49. *R. alismaefolius*.
- Receptacle elongated by 1 or 2 mm. (rarely more) in fruit; stems 1–4 dm. long; leaves entire.
  - Achenes glabrous; herbage glabrous.
    - Leaves lanceolate; petals about 10 mm. long .... 49A. Var. *Hartwegii*.
    - Leaves ovate or ovate-lanceolate; petals usually about 6 mm. long .... 49B. Var. *alismellus*.
  - Achenes pubescent or glabrate; herbage pilose or glabrous; petals 8–16 mm. long; leaves lanceolate .... 49C. Var. *Lemmonii*.

Petals 10 or rarely 5–9, oblanceolate or sometimes broadly so . . . 49D. Var. *montanus*.

49A. RANUNCULUS ALISMAEFOLIUS var. HARTWEGII (Greene) Jepson, Fl. Calif. **1**: 534. 1922. *R. alismaefolius* var. *caule petiolisque basi hirsutis* Benth. Pl. Hartw. 295. 1848. *R. Hartwegii* Greene, Erythea **3**: 45. 1895. *R. arnoglossus* Greene, Pittonia **4**: 143. 1900.

Glabrous; stems 2–4 dm. long, 1.5–2.5 mm. in diameter, branching above, several-flowered; radical leaf blades lanceolate, 4–10 cm. broad, entire, of intermediate thickness; petals 5, obovate, 6–8 mm. long, the nectary scale attached nearly to the apex, often lobed; achenes 20–30, each 2 mm. long, 1.5 mm. dorsoventrally, 0.8 mm. laterally, the beak about 0.7 mm. long; receptacle 1–1.5 mm. long in fruit.

Mountain meadows at 1,400–2.200 meters elevation; mountains of central Oregon to the Sierra Nevada as far south as Calaveras County, California, and to Southern Idaho; Washoe and Ormsby Counties and the East Humboldt Mountains, Nevada; Helena and Midvale, Montana; Yellowstone National Park and Leckie, Wyoming. Western pine forest. May and June.

Occurring in the Oregon Cascades as an intergrade toward var. *alismellus*.

Type collections: (1) Var. *caule petiolisque basi hirsutis*, "In uliginosus (Bear Valley) montium Sacramento," *Hartweg 1627* (*155*), Sierra Nevada in Nevada County, California. (2) *R. Hartwegii*. Based upon the unnamed variety listed above. (3) *R. arnoglossus*, "Subalpine in the Ruby [East Humboldt] Mountains, eastern Nevada; collected by the writer [Greene], 20 July, 1896." The TYPE is *HGr. 2388–9*.

49B. RANUNCULUS ALISMAEFOLIUS var. ALISMELLUS A. Gray, Proc. Am. Acad. **7**: 327. 1868. *R. alismellus* Greene, Fl. Fran. **1**: 297. 1892.

Glabrous; roots 6–12 slender; stems 1–2 or 3 dm. long, 1–1.5 mm. in

diameter, often simple, usually 1- or 2-flowered; radical leaf blades ovate-lanceolate or sometimes ovate, 2–4 cm. long, entire, thin; petals 5, obovate, about 6 mm. long, the nectary scale attached laterally usually almost to the apex, truncate or rounded; achenes 10–30, each 1.5 mm. long, 1.2 mm. dorsoventrally, about 0.5 mm. laterally; receptacle about 1 mm. long in fruit.

Mountain meadows and wet ground near snow banks at 1,400–2,000 meters elevation northward and 2,200–3,300 meters southward; eastern peaks of the Cascade Mountains in central and southern Washington; high mountains of Oregon to the high North Coast Ranges and the San Jacinto Mountains, California; near Virginia City and in the East Humboldt (Ruby) Mountains, Nevada. Mostly northern coniferous forest. June and July.

The Oregon form is more robust and with branching stems, and it is an intermediate toward the variety *Hartwegii*. Typical var. *alismellus* occurs on the eastern side of the Cascade Mountains in Washington.

Type collection: "Lake Tenayo [Tenaya] and on Mt. Dana, Sierra Nevada, to the height of 12,000 feet, Bolander." Yosemite National Park, California. The Lake Tenaya specimen is *Brewer 1684*. Only the Mt. Dana specimen is by Bolander, and it is designated as a LECTOTYPE, *GH*.

49C. RANUNCULUS ALISMAEFOLIUS var. LEMMONII (A. Gray) L. Benson, Am. Jour. Bot. **23**: 172. 1936. *R. Lemmonii* A. Gray, Proc. Am. Acad. **10**: 68. 1875.

Commonly pilose at least on the stems and petioles; stems decumbent, 1.5–3 dm. long, 1.5–3 mm. in diameter; radical leaves lanceolate, 3–9 cm. long, 5–10 mm. broad, entire; petals 5, obovate, 8–16 (mostly 10) mm. long, the nectary scale like the typical species; achenes about 20, each about 2 mm. long, 1.8 mm. dorsoventrally, about 0.8–1 mm. laterally, pubescent, glabrate, or glabrous, the beak 0.5–1 mm. long; receptacle pyriform, 2–5 mm. long in fruit.

Meadows of valleys in the arid mountain region at 1,500–2,000 meters elevation; Modoc County to Truckee, California. Western pine forest. May to July.

Type collection: "Sierra Valley, California, alt. 5,000 feet, J. G. Lemmon." The TYPE is in the Gray Herbarium. The Sierra Valley specimens (apparently isotypes) seen in other herbaria are not this variety. In fact, three or four species are represented among them.

42D. RANUNCULUS ALISMAEFOLIUS var. MONTANUS S. Wats. Rept. U. S. Geol. Expl. 40th. Par. **5**: 7. 1871. *R. calthaeflorus* Greene, Erythea **3**: 45. 1895. *R. alismaefolius* var. *calthaeflorus* Davis, Minn. Bot. Studies **2**: 495. 1900. *R. unguiculatus* Greene, Pittonia **4**: 142. 1900.

Glabrous; stems erect or reclining at the bases, branching above, and the inflorescence cymose but flat-topped, as it is also in *R. alismaefolius* and var. *Hartwegii;* radical leaf blades narrowly elliptic, 2–6 cm. long, 1–2.5 cm. broad, entire, of intermediate thickness, proximally tapering and distally acute; petals 10 (rarely 5–9), 5 or commonly 7–12 mm. long, 2–3 or rarely 5 mm. broad, usually oblanceolate, proportionately much narrower than in

any of the other varieties, the nectary scale forming a broad pocket 0.4–0.5 mm. deep; achenes 25–60, each about 2 mm. long, 1.3 mm. dorsoventrally, 1 mm. laterally; receptacle 2–3 mm. long in fruit.

Mountain meadows at 2,700–3,000 meters; East Humboldt (Ruby) Mountains, Nevada, to Western and Southern Wyoming and Colorado. Northern coniferous and western pine (lodgepole pine) forest. June and July.

Type collections: (1) Var. *montanus,* "Shore of Marian Lake in the East Humboldt [Ruby] Mountains, Nevada, and at the head of the Provo River in the Uintas; 9,000 feet altitude; June–August. (18.)" The collection from the Uintah Mountains by Sereno Watson in 1868 is designated as a LECTOTYPE, GH. In the U. S. National Herbarium the Marian Lake specimen is sheet *No. 862,* and the Uintah plant is mounted on the same sheet (unnumbered). (2) *R. calthaeflorus,* "Plant of the Colorado Rocky Mountains chiefly, at elevations a little below the limit of trees; the *R. alismaefolius,* var. *montanus* of S. Watson, partly; also the type of the unpublished *R. alismaefolius,* of Geyer." At the bottom of the same page, Greene states, "Geyer's specimens, on the labels of which he wrote this [*R. alismaefolius*] as a new name were of the Rocky Mountain species which I now name *R. calthaeflorus.*" Greene confuses Geyer's specimens from the "Rocky Mountains," but actually from Coeur d'Alene, Northern Idaho, with the plants from the southern and central Rocky Mountain System, which is the one he describes as *R. calthaeflorus.* (Note that the petal number is given as 10). That Greene did not know Geyer's plant differed from Watson's variety *montanus* is emphasized further by the following statement, "*R. Hartwegii* of the Californian Sierra differs essentially from the Rocky Mountain *R. calthaeflorus* . . . in its broadly obovate petals only half as numerous, namely five." So also does Geyer's plant (cf. isotype, *S*), which is absolutely identical with Greene's *R. Bolanderi* from coastal California. However, the following is quoted from Greene, "Asa Gray's statement that '*R. Bolanderi,* Greene, Bull. Calif. Acad. ii. 58, answers to the type of this species [*R. alismaefolius*]' is not only without foundation; it proves that the author did not know that Geyer's type was not Californian." What really is proved is that Greene did not understand Geyer's type and knew only that it came from the "Rocky Mountains" and that he was really describing the southern and central Rocky Mountain plant as *R. calthaeflorus,* and not renaming Geyer's species. Several specimens collected prior to 1895 are in the Herbarium Greeneanum and *No. 2391* of that herbarium is designated as a LECTOTYPE of *R. calthaeflorus.* It was collected in wet, boggy ground near Georgetown, Colorado at 12,000 feet altitude by *Chas. S. Sheldon* on August 17, 1884, *No. 281.* (3) *R. unguiculatus,* "Common 'on sites of old snow banks' at 11,500 feet in the mountains of southern Colorado, C. F. Baker, 28 Aug., 1899." The TYPE is *HGr. 2994 and 7801.*

50. RANUNCULUS ORESTERUS L. Benson, apud Benson & Carter, Am. Jour. Bot. **26**: 555. 1939.

Swales at 1,300 meters elevation in the Blue Mountains between Baker and Canyon City, Eastern Oregon; New Meadows, Adams County, Idaho (*Ray J. Davis 94, I S B*), cf. L. Benson, Am. Jour. Bot. **27**: 187–8. 1940. Western pine forest. June.

Type collection: "In swales at the summit of the Blue Mountains, Eastern Oregon, altitude 4,000 feet (fide Washington State College specimen), Baker City-Canyon City road. Known only from the type collection by *William C. Cusick,* June 4, 1902, *No. 2800.* Type specimen in the Dudley Herbarium, Stanford University, California, *No. 96453.* Isotypes: University of Oregon Herbarium, 3 sheets marked by the writer in 1932 with a square containing an x in its lower left corner; Washington State College Herbarium *No. 10201;* New York Botanical Garden." One of the specimens from the University of Oregon was presented to the herbarium of the writer in 1939 through the courtesy of Dr. LeRoy Detling.

51. RANUNCULUS AMBIGENS S. Wats. Bibl. Ind. N. Amer. Bot. **1**: 16. March, 1878 and Proc. Am. Acad. **14**: 289. May 14, 1879. (?) *R. obtusiusculus* Raf. Med. Repos. N. Y. II. **5**: 359. 1808, *nomen dubium,* cf. Fern. Rhodora **38**: 173–5. 1936. *R. Flammula* var. *major* Hook. Fl. Bor. Amer. **1**: 11. 1829. *R. ambigens* var. *obtusiusculus* Davis, Minn. Bot. Studies **2**: 494. 1900, *nomen dubium.*

Wet clay soil at low altitudes; Minnesota to Maine (York) and southward to Louisiana, Tennessee, southeastern Virginia (Appomattox River), and possibly Georgia (Chapman). Mostly coastal in New England. Largely hardwood forests. May to August.

Type collections: (1) *R. obtusiusculus,* "In New-Jersey in marshy places." Observed in 1803 and 1804 by C. Rafinesque-Schmaltz. Fernald, *loc. cit.,* writes as follows, "That Rafinesque's drawing of an *annual,* with bluntish leaves, leafy-bracted peduncles, gamopetalous corolla, linear or linear-lanceolate sepals, and rounded obovate petals (his fig. 2), is not a recognizable illustration of *R. ambigens,* which is a coarse and obvious perennial, with attenuate leaves, bractless peduncles, ovate sepals and distinct (as in all the genus) oblong petals, should be obvious. . . . Except for the alternate leaves the drawing of the habit could as well have been made from a vague recollection of *Lysimachia (Steironema) lanceolata* as from *Ranunculus ambigens.*" (2) Var. *major,* "Canada. *Mr. Goldie.*" (3) *R. ambigens,* as first published (Bibl. Ind. N. Amer. Bot. **1**: 16. March, 1879) "In inundated places and small rivulets: Pennsylvania & Virginia."

52. RANUNCULUS FLAMMULA L. Sp. Pl. 548. 1753.

Stems reclining, sometimes stoloniferous, rooting adventitiously at the lower nodes, 1–5 dm. long and 2–7 mm. in diameter, branching above and 2–25-flowered, fistulous below, glabrous or with a few stout, appressed hairs; leaves alternate, the blades simple, mostly oblanceolate, or lanceolate or sometimes ovate-lanceolate, 2–6 or 8 cm. long, 3 or 5–10 or 13 mm. broad, entire or serrulate, acute at both ends, glabrous or somewhat appressed-hairy, the petioles mostly 2–7 cm. long; petals 5, 4–8 mm. long, 4–7 mm. broad; stamens 25–50; achenes 20–50 in a globose head 3–5 mm. long and 4–5 mm. in diameter, each achene obovate, 1.4–1.7 mm. long, 1–1.2 mm. dorsoventrally, the achene beak stout, 0.1–0.2 mm. long, straight.

Marshy ground near sea level; Europe; Quiddy Viddy Lake, Newfoundland, and Yarmouth County, Nova Scotia; occurring on the Pacific Slope (Washington and Oregon) as an intergrade to var. *ovalis.* Northern coniferous forest. July to early September.

Type collection: "Habitat in Europae *pascuis udis."*

No one of the three following varieties is wholly clear in its definition. However the populations are well-marked in extreme forms:

52A. RANUNCULUS FLAMMULA var. **ovalis** (Bigel.) L. Benson, comb. nov. *R. filiformis* Michx. var. *ovalis* Bigel. Fl. Bost. Ed. 2. 239. 1824, not *R. ovalis* Raf. in 1814. *R. reptans* L. var. *ovalis* Torr. & Gray, Fl. N. Am. **1**: 16. 1838. *R. unalaschensis* Bess. ex Ledeb. Fl. Ross. **1**: 32. 1842, as syn. *R. Flammula* L. var. *unalaschensis* Ledeb. Bull. Soc. Nat. Mosc. **34**[2]: 41. 1861. *R. reptans* L. var. *strigulosus* Freyn. Deuts. Bot. Monatss. **8**: 181. 1890. *R. microlonchus* Greene, Erythea **4**: 122. 1902. *R. Flammula* var. *varians* Blankinship, Mont. Agric. Coll. Sci. Studies Bot. **1**: 55. 1905. *R. Flammula unalaschensis* Piper, Contr. U. S. Nat. Herb. **11**: 272. 1906. *R. Flammula* L. var. *strigulosus* Freyn ex Peck, Man. Higher Pl. Ore. 302. 1941, nomen nudum.

Stems 1–4.5 dm. long, mostly 0.8–1.5 mm. in diameter, not necessarily rooting at all or any of the nodes; radical leaf blades markedly broader than the petioles, 1–5 cm. long, 1.5–7 or rarely 13 mm. broad, broadest at the middles and tapering to both ends, entire, the petioles 2–6 or up to 13 cm. long; cauline leaves 1–2 or 3 cm. long, 1.5–4 or 6 mm. broad, oblanceolate or broadest at the middles; petals 5, commonly 3.5–5 or rarely 2 or 6.5 mm. long; achene beak 0.3–0.6 mm. long; intermediate in quantitative characters between *R. Flammula* and var. *filiformis.*

Muddy or marshy ground or wet sand from sea level up to 1,000 meters elevation northward or up to 2,500 meters southward; from Alaska southward to north-coastal and montane California, Arizona (Kaibab Forest), and Colorado and eastward to Newfoundland and New England. Summer.

This variety is best-developed in the Western States, especially in the Pacific States. The New England and New York form, to which the type (Boston) must have belonged is really an intermediate between the extreme of var. *ovalis* and var. *filiformis.* For this reason, the name var. *strigulosus* has been previously maintained for the Western form. However, the extreme form occurs here and there in eastern Canada and in the northern tier of states from Minnesota eastward. A form with unusually short, broad, thick radical and cauline leaves occurs in Newfoundland (St. John Bay, *Fernald et al. 28256,* GH; Ingornachoix Bay, *Fernald & Wiegand 3413,* GH.)

Type collections: (1) Var. *ovalis,* "Sent from Danvers by Mr. Nichols." Mr. Weatherby of the Gray Herbarium wrote as follows on January 10, 1939, "We have none of Bigelow's original material of *R. reptans* var. *ovalis.* Whether any exists, I do not know. I have no doubt, however, that the material we have represents this plant." The common, rather broad-leaved form from the vicinity of Boston is accepted as similar to the type. (2) *R. unalaschensis, "R. unalaschensis,* Besser in herb. Zeyheri." "Hab. in Unalaschka! (Chamiss., Eschsch.)" This plant was interpreted by Fernald, Rhodora **19**: 137. 1917. as *R. reptans* var. *ovalis.* The type has not been seen. (3) Var. *strigulosus,* "Oregon. Mt. Hood. 24 Juli 1888, leg. Röll." Peck in combining this varietal name under *R. Flammula* gave no reference to Freyn's com-

bination under *R. reptans*. (4) *R. microlonchus*, "Collected by the writer in northern Idaho, August, 1889." Lake Pend Oreille, Greene, Aug. 9, 1889. Type, *HGr 2423*. (5) Var. *varians*, "Crow Creek, Mission Mts. Montana, Aug., 1897, M. J. Elrod, 234." It is possible that the new combination may be published first in Abrams, Ill. Fl. Pac. Sts. vol. 2.

52B. RANUNCULUS FLAMMULA var. FILIFORMIS (Michx.) Hook. *R. reptans* L. Sp. Pl. 549. 1753. *R. filiformis* Michx. Fl. Bor. Am. **1**: 320. 1803. *R. reptans* var. *filiformis* DC. Syst. **1**: 248. 1818. *R. Flammula* var. *intermedia* Hook. Fl. Bor. Am. **1**: 11. 1829, in part. *R. Flammula* var. *filiformis* Hook. Fl. Bor. Am. **1**: 11. 1829. *R. Flammula* var. *reptans* E. Mey. Pl. Lab. 96. 1830. *R. reptans* var. *intermedius* Torr. & Gray, Fl. N. Am. **1**: 16. 1838, in part. *R. intermedius* Heller, Bull. Torrey Club. **25**: 580. 1896, neither Poir. in 1804 nor. Eat. in 1822.

Stems stoloniferous, reclining, rooting adventitiously, 1–3 or rarely 4 dm. long and 0.2–1 mm. in diameter, rarely larger in the lowest internode, branching or unbranched, radical leaves simple, filiform or linear, not or else barely expanded into a blade, 1.5–6 or 9 cm. long, 0.5–1.5 mm. broad, entire, distally truncate with a differentiated (callus or glandular ?) apex, glabrous or with stout, appressed hairs about 2–8 mm. long; cauline leaves in clusters at the nodes where roots enter the soil, actually alternate, the blades expanded, tapering to both ends, 3 mm. long, 1–1.5 or 2 mm. broad, petioled; petals 5 or up to 11, 2–4 or rarely 7 mm. long, 1.3–2.5 or rarely 3.5 mm. broad; stamens 5–25; achenes about 5–15 in a hemispherical cluster 1.5–2.5 mm. long by 2–3.5 mm. in diameter, each achene 1.3–1.6 mm. long, 1.1–1.2 mm. dorsoventrally, the achene beak slender above, 0.3–0.5 or rarely 0.2 or 0.7 mm. long, recurved.

Marshy ground of lakes, streams, and ditches from sea level (northward) to 2,000 meters elevation (southward); Northern Europe and Northern Asia; Alaska to Labrador and Greenland and southward to Bull Lake Creek, Wyoming, and the northern tier of states from Minnesota eastward; New England; Susquehanna River in northeastern Pennsylvania. Northern coniferous and Northeastern hardwood forests. Summer.

Type collections: (1) *R. reptans*, "*Habitat* in Suecia." (2) *R. filiformis*, "HAB. *ad ripas* S. Laurentii et sinum Hudsonis." (3) Var. *intermedia*, "On gravelly banks of rivers from Canada to lat. 60°. *Dr. Richardson. Drummond.* Newfoundland. *Mr. Morrison.*" The description was as follows: "*intermedia;* caule repente gracili, foliis anguste lanceolatis superioribus linearibus integerrimus." According to Fernald, Rhodora **19**: 137. 1917, "It is clear that Hooker was merely separating from the true slender-leaved *R. reptans* (his *R. Flammula filiformis*) a broader-leaved but repent slender plant of Canadian river banks, a plant scarcely separable from *R. reptans*, but somewhat broader-leaved than the typical form of the species."

52C. RANUNCULUS FLAMMULA var. **samolifolius** (Greene) L. Benson, comb. nov. *R. samolifolius* Greene, Pittonia **3**: 13. 1900. *R. reptans* var. *samolifolius* L. Benson, Am. Jour. Bot. **23**: 171. 1936.

Stems 1–4 dm. long, rather stout; leaf blades ovate to obovate or very broadly oblanceolate, 1.5–3 cm. long, 1–1.2 cm. broad, usually sessile and the petioles usually sheathing the stem; otherwise like var. *ovalis*.

Mountain meadows at 1,500–2,000 meters elevation; Cascade Mountains from Klamath County, Oregon to Plumas County, California. Northern Coniferous and Western Pine Forests. Summer.

A fairly well-marked geographical form, the characters of which barely entitle it to varietal rank.

Type collection: "A well-marked species of the higher Sierra Nevada, Calif., from Mt. Shasta southward." The following specimen from the only definite locality mentioned is designated as a LECTOTYPE: Mt. Shasta, *C. H. Dinwiddie* in 1883, *HGr. 2456*. It represents the extreme form of the variety. Publication of this variety in Abram's Illustrated Flora of the Pacific States Vol. 2 may precede publication here.

Significant specimens, cf. L. Benson, Am. Jour. Bot. **23**: 171. 1936.

53. RANUNCULUS HYDROCHAROIDES A. Gray, Mem. Am. Acad. II. **5**: 306. 1855.

Glabrous aquatic or palustrine perennials; stems procumbent or floating or some of them suberect, rooting at the lower nodes, 10–25 cm. long and 1.5–4 mm. in diameter, 1–3-flowered, often fistulous; radical leaf blades simple, ovate, 1.5–3.5 cm. long, 1–2.8 cm. broad, entire, proximally truncate or rarely subcordate and distally usually acute, petioles 2–3.5 cm. long, stipular leaf bases 0.5–1 cm. long; cauline leaves alternate, like the radical, petioled; petals 5.

Marshes, streams, and springs at 2,200 or 2,400 meters; Owens Lake (undoubtedly the Sierra Nevada above it), California; Flagstaff, the White Mountains, and southeastern Arizona; southwestern New Mexico; northwestern Mexico. Western pine forest. June to September.

Type collection: "In wet marshes, Mabibi, Sonora; June." *Thurber.*

53A. RANUNCULUS HYDROCHAROIDES var. STOLONIFER (Hemsl.) L. Benson, Am. Jour. Bot. **27**: 187. 1940. *R. stolonifer* Hemsl. Diagn. Pl. Nov. 17. 1879.

Palustrine; flowering stems erect, 1–3 dm. long, slightly fistulous, stolons 2–4 dm. long; radical leaf blades cordate or ovate, 0.7–3.7 cm. long, 0.4–3 cm. broad, dentate; cauline leaves narrowly elliptic, 1.5–3 cm. long, 0.2–0.8 cm. broad, usually dentate; petals perhaps sometimes more than 5.

Marshy ground of springs and streams in the mountains at about 2,000 meters elevation; Eastern Arizona; New Mexico; Mexico. Western pine forest. Summer.

Type collection: "Mexico, in regione San Luis Potosi, alt. 6000–8000 ped., Perry et Palmer, 4. (Hb. Kew.)"

54. RANUNCULUS GORMANII Greene, Pittonia **3**: 91. 1896. *R. reptans* L. var. *Gormanii* Davis, Minn. Bot. Studies **2**: 498. 1900. *R. terrestris* Wynd, Torreya **30**: 53. 1930.

Boggy mountain meadows at 2,000–2,500 meters; Cascade Mountains from the Three Sisters, Oregon, to the Siskiyou and Klamath Mountains, California. Northern coniferous forest. June and July.

Type collections: (1) *R. Gormanii*, "On moist banks at Cathedral Springs, Crater Lake, in southern Oregon, 22 Aug., 1896, collected by Mr. M. W. Gorman." The TYPE is *HGr. 2408*. (2) *R. terrestris*, "The type has been deposited in the Herbarium of the University of Oregon as:—*Wynd, no. 2086*, Red Blanket Creek in the southwest corner of Crater Lake National Park."

Significant specimens, cf. L. Benson, Am. Jour. Bot. **23**: 172. 1936; Madroño **2**: 129. 1934.

55. RANUNCULUS POPULAGO Greene, Erythea **3**: 19. 1895. *R. Cusickii* Jones, Proc. Calif. Acad. II. **5**: 615. 1895. *R. alismellus* (A. Gray) Greene var. *Populago* Davis, Minn. Bot. Studies **2**: 496. 1900.

Mountain meadows at 1,500–2,000 meters elevation; Blue Mountains, Washington, to the Bitterroot Mountains, Idaho, and the Siskiyou Mountains and Butte County, California. Northern coniferous forest. Summer.

Type collections: (1) *R. Populago*, "A somewhat rare plant of the mountains of eastern Oregon and adjacent Idaho, distributed by Mr. Cusick and others under various names. . . ." Greene, Pittonia **3**: 14. 1896, added the following statement, "RANUNCULUS POPULAGO, Greene, Eryth. iii, 19, has a synonym in *R. Cusickii*, Jones, Proc. Calif. Acad. n. ser. v. 615. Cusick's number 1161 is also my type of the species." The TYPE is *HGr. 2756*. (2) *R. Cusickii*, "The type is No. 1161, Cusick, Eagle Co., Or., 1884, 6000° alt."

Significant specimens, L. Benson Am. Jour. Bot. **23**: 171. 1936; Madroño **2**: 129–130. 1934.

56. RANUNCULUS LAXICAULIS Darby, Bot. S. Sts. 204. 1855. *R. Flammula* L. var. *laxicaulis* Torr. & Gray, Fl. N. Am. **1**: 16. 1838. *R. pusillus* var. *denticulatus* Torr. & Gray, Fl. N. Am. **1**: 17. 1838. *R. texensis* Engelm. apud Engelm. & Gray, Bost. Jour. Nat. Hist. **5**: 210. 1847.

Stems erect or reclining, rooting adventitiously at only the lowest nodes, 1–5 dm. long and 1–5 mm. in diameter, freely branching, fistulous; radical leaf blades simple, oblong to ovate-obtuse, 1–4 cm. long, 6–18 cm. broad, dentate or entire, proximally truncate or rounded and distally truncate or obtuse, petioles 1–7 cm. long, stipular leaf bases 0.5 to about 1 cm. long; petals 5, 3–4 or 7 mm. long, 1.5–2 mm. broad.

Marshy ground and ditches at low elevations; lowlands near the Mississippi River system from Missouri, southern Illinois, and northwestern Kentucky southward to southeastern Texas and thence eastward to northeastern Georgia and northward on the coastal plain to Maryland (Snow Hill) and Delaware (Laurel). River bottom forest. April to June.

Long known as *R. oblongifolius* Ell., which is a synonym for *R. pusillus* Poir.

Type collections: (1) Var. *laxicaulis*, "Milledgeville, Georgia, *Dr. Boykin!*" The writer has not located the Boykin specimen at the New York Botanical Garden or elsewhere. Dr. Fernald, Rhodora **38**: 173–5. 1936, writes as follows concerning applicability of the name *R. laxicaulis* to *R. ambigens* S. Wats. "The Boykin specimen, type of *R. Flammula* β *laxicaulis* is not at the Gray Herbarium and Dr. Gleason writes me that it cannot be

found in the Herbarium of the New York Botanical Garden. Nor have I seen in either herbarium any material [of *R. ambigens*] from the Atlantic States from south of Delaware or Maryland, although there is a specimen without detailed data at New York said on the copied label to be from Georgia. This, however, is one of the many unlocalized sheets from Chapman, too many of which are open to doubt. The petals of *R. ambigens* only slightly exceed the sepals (sepals 5–7 mm. long, petals 5–8 mm. long); but Torrey & Gray described the 'petals . . . three times as long as the calyx.' They also had 'a weak much branched' plant with 'leaves all entire,' not a convincing description of the coarse stem (0.5–2 cm. thick at the base), simple or slightly forking, of *R. ambigens*, which has the middle and upper leaves toothed. Their description suggests *R. oblongifolius* Ell.; at least it is unwise to maintain *R. laxicaulis* for the undoubted *R. ambigens*." Fernald, Rhodora **41**: 541–2. 1939, argued further as follows: "Both the original Torrey & Gray account of Boykin's plant and the fuller account by Darby are perfect descriptions of the plant erroneously passing as *R. oblongifolius*. This I intimated in 1936 (Rhodora, xxxviii. 175), when I showed that the name *R. laxicaulis* certainly does not belong to *R. ambigens* Wats. Although, as then stated, the type can not be found, the descriptions are so convincing to one who has followed through the surrounding vegetation the 'weak, much branched, declined' stems, 'rooting at the lower joints,' that I now have no hesitation in taking up for *R. oblongifolius* of authors, not Ell. the appropriate name R. LAXICAULIS (T. & G.) Darby." (2) Var. *denticulatus*, "β Texas, *Drummond*." (3) *R. texensis*, "Margin of ponds, &c. near Houston. April." *F. Lindheimer*. "The collection of 1843 was made on Galveston Island, around Houston, on the Brazos, &c." The specimen at the Gray Herbarium is from Houston, April 1842, legit Lindheimer 1843.

56A. RANUNCULUS LAXICAULIS var. **mississipiensis** (Small) L. Benson, comb. nov. *R. mississipiensis* Small, Bull. Torrey Club **27**: 277. 1900.

Stems 3 or 5–6 mm. in diameter; basal leaves ovate-acute, 3 cm. long, 2 cm. broad, strongly dentate; petals 6–10, 5–9 mm. long, 2–2.5 mm. broad.

Marshy ground at low elevations; Lincoln County (Varner) and Ashley County (Mist.), Arkansas, and "Alluvions of Mississippi." River bottom forest. Early spring.

A rather poorly differentiated variety.

Type collection: "ARKANSAS: Varner, Lincoln Co., April 28, 1898; *Bush*, no. 12. MISSISSIPPI: 'Alluvions.' 1840; *Peck*." In a conversation with the writer at the New York Botanical Garden about August 1, 1935, Dr. Small designated the Mississippi "alluvions" specimen at the New York Botanical Garden as the TYPE. According to the writer's note, it was collected by Short.

57. RANUNCULUS PUSILLUS Poir. in Lam. Encyc. Meth. **6**: 99. 1804. *R. humilis* Pers. Syn. **2**: 102. 1807. *R. oblongifolius* Ell. Sketch. **2**: 58. 1816. *R. pusillus* var. *muticus* Torr. & Gray, Fl. N. Am. **1**: 17. 1838. *R. pusillus* var. *oblongifolius* Torr. & Gray, Fl. N. Am. **1**: 17. 1838. *R. trachyspermus* Engelm. var. *Lindheimeri* Engelm. apud Engelm. & Gray, Bost. Jour. Nat. Hist. **5**: 211. 1847. *R. pusillus* var. *Lindheimeri* A. Gray, Proc. Am. Acad. **21**: 367. 1886. *R. Biolettii* Greene, Pittonia **2**: 225. 1892.

Radical and lower cauline leaf blades simple, oblong to ovate or rarely cordate, distally truncate or rounded; sepals 1–1.5 mm. long; achenes 15–50 in a hemispherical head about 4 mm. in diameter or an ovoid head 2–4 mm. long by 2–2.5 mm. in diameter, each achene oblong-obovate, 1 mm. long, 0.7 mm. dorsoventrally, 0.3 mm. laterally, smooth or slightly or markedly papillate, glabrous, margin inconspicuous, the style in anthesis 0.1–0.2 mm. long, the achene beak 0.1–0.2 mm. long, receptacle pyriform or spherical, 1.5–2 mm. long in flower and 1.5–3 mm. long in fruit, glabrous.

Shallow water of ditches and marshy ground at low elevations; Missouri to New York, southeastern Texas, and Georgia. April to June.

The form occurring in the Gulf States has strongly-papillate achenes. It has been called var. *Lindheimeri.* This form is either native or naturalized in the seaward Coast Ranges of California from Humboldt County to the Santa Cruz Mountains and eastward to Napa County.

Type collections: (1) *R. pusillus,* "Cette plante croît dans la Caroline, dans les lieux humides & marécageux. Elle m' a été communiquée par M. Bosc, qui l'y a recuellie." (2) *R. humilis,* "*R. pusillus* Poir. enc. bot. 6. p. 99. *R. Flammula* Walter. Hab. in Carol. humilis." (3) *R. oblongifolius,* "Collected 12 miles from Savannah on the Augusta road. St. John's Berkley. Dr. Macbride." According to Fernald, Rhodora **41**: 542. 1939, ". . . When Mr. Long and I studied Elliott's material at Charleston in early April, . . . we found that the type of *R. oblongifolius* is characteristic large material of *R. pusillus.* . . ." (4) Var. *muticus,* "New York! to Pennsylvania." The specimen in the New York Botanical Garden, labelled Torrey, Flora of New York (abbreviated) is designated as a LECTOTYPE. (5) Var. *Lindheimeri,* "Near Houston, etc. but not growing together with No. 2 [*R. trachyspermus*]," *Lindheimer* in 1843. (6) *R. Biolettii,* "On Hood's Peak, Sonoma Co., California, May 1, 1889, Mr. F. T. Bioletti."

57A. RANUNCULUS PUSILLUS var. **angustifolius** (Engelm.) L. Benson, comb. nov. *R. trachyspermus* Engelm. apud Engelm. & Gray, Bost. Jour. Nat. Hist. **5**: 211. 1847, not Ell. in 1816. *R. trachyspermus* var. *angustifolius* Engelm. apud Engelm. & Gray, Bost. Jour. Nat. Hist. **5**: 211. 1847. *R. tener* Mohr, Contr. U. S. Nat. Herb. **6**: 513. 1901.

Lower leaves commonly distally acute; sepals 1.5–2 mm. long; achenes 50–125, rarely fewer, in a cylindrical head usually 5–8 mm. long, each achene densely and conspicuously papillate; receptacle cylindrical, 4–7 mm. long in fruit.

Ditches and marshy ground; southeastern Texas; Auburn and Mobile, Alabama. River bottom forest. April to June.

Type collections: (1) *R. trachyspermus,* "Margin of ponds near Huston, &c.," *Lindheimer.* The specimen in the Gray Herbarium bearing Engelmann's label was collected in April 1842, *legit* Lindheimer in 1843; Houston, Texas. It is numbered 2. (2) Var. *angustifolius,* "Near Houston, etc. but not growing together with No. 2 [*R. trachyspermus*]," *Lindheimer* in 1843. The specimen is number 3. (3) *R. tener. Nomen novum* for *R. trachyspermus.*

58. RANUNCULUS ALVEOLATUS Carter, apud Benson & Carter, Am. Jour. Bot. **26**: 555. 1939.

Margins of ponds and marshy areas along small streams up to 1,000 meters elevation; Sierra Nevada foothills from Calaveras County to Placer County, California. Oak woodland. April and May.

Type collection: "The type specimen was collected between Fair Oaks and Folsom, 1½ miles southeast of Orangevale. Sacramento County, April 25, 1937, *Annetta Carter 1244* (*Herbarium of the University of California No. 604080.*)"

SECT. 5. HECATONIA (LOUR.) DC.

Styles and achene beaks practically lacking, the stigmas nearly sessile; achene with or without corky thickening of the pericarp.
- Achenes smooth on the faces; receptacle not more than 5 mm. long in fruit; perennials; stems rooting at the nodes.
  - Leaves not cordate at the bases; achenes 10–20; receptacle but slightly enlarged in fruit, 1.5 mm. long . . . . 58. *R. hyperboreus*
  - Leaves (at least some of them) cordate at the bases; achenes 20–60; receptacle greatly enlarged in fruit, 3–5 mm. long . . . . 59. *R. natans*
- Achenes marked on each face by either minute rough transverse ridges or by a circle of "pin pricks" at the inner margin of the peripheral pericarp thickening; annuals; stems never rooting adventitiously except in one rare variety; receptacle greatly enlarged in fruit, 2.5–9 mm. long . . . . 60. *R. sceleratus.*

Styles and achene beaks well-developed, the beaks at least half as long as the achene bodies, 0.6–1.5 mm. long; achenes with conspicuous corky thickening of either the keel or the pericarp beside the keel.
- Achenes each with corky thickening beside the inconspicuous keel (especially in the basal and ventral regions); leaves once- or twice-parted or -lobed, pentagonal, 1–2 cm. long by 1.5–2.5 cm. broad; anthers elliptic, 0.5–1 mm. long; petals 4–7 mm. long . . . . 61. *R. Gmelinii.*
- Achenes each with a conspicuous corky keel; leaves of aquatic specimens finely dissected into ribbon-like segments 1–2 mm. broad, the blades 1.5–10 cm. long and 2–12 cm. broad; anthers oblong, 1–1.5 mm. long; petals 7–15 mm. long . . . . 62. *R. flabellaris*

58. RANUNCULUS HYPERBOREUS Rottb. Skrift. Kjoeb. Selsk. **10**: 458. 1770. *R. hyperboreus* f. *fluitans Porsild,* Meddel. Groenl. **50**: 375. 1912.

Marshy ground in boreal regions near the sea; circumboreal; from Alaska along the shore and islands of the Arctic Ocean to Greenland; Old Hollowtop, near Pony, Montana. Arctic-Alpine Grassland. July to August.

Type collection: (1) *R. hyperboreus*, "Island." The title of the Article is "Rare Planter som i Island of Grønland." (2) F. *fluitans,* "Neighborhood of Prøven 7 the northern district of Umanaq . . . occurs in pools."

59. RANUNCULUS NATANS C. A. Mey. in Ledeb. Ic. **2**: 114. 1830. *R. hyperboreus* Wahl. var. *natans* Regel, Reisen Ost-Sib. **1**: 43. 1861. *R. intertextus* Greene, Ottawa Nat. **16**: 33. 1902.

Ponds and lakes at 2,000–2,700 meters elevation in the Central Rocky Mountains and at lower elevations northward; Siberia; Nordegg; Alberta;

Custer County and Beaver Canyon, Idaho; Wyoming and Colorado. Northern Coniferous Forest. July and August.

Type collections: (1) *R. natans*, "Hab. in aquis stagnatibus ad fl. Tscharysch, in fl. Mön et Tegagon in fl. Tschiya influentibus." Flora Rossicam-Altaicam. (2) *R. intertextus*, "Common almost throughout the Rocky Mountains, as an aquatic of subalpine ponds and swamps." "Hitherto referred to *R. natans* of Europe."

60. RANUNCULUS SCELERATUS L. Sp. Pl. 551. 1753.

Stems erect, never rooting adventitiously, 1–10 dm. long and 2–15 mm. in diameter; radical leaf blades simple, reniform, 1–3 or 6 cm. long, 1.5–5 or 10 cm. broad, deeply 3-parted or -divided, the primary parts or divisions usually merely lobed, but sometimes parted or divided, the ultimate lobes obtuse, the sinuses rounded, proximally cordate and distally rounded, petioles 3–12 cm. long, stipular leaf bases 5–10 mm. long, broad; each achene obovate, 0.8–1 mm. long, 0.6 mm. dorsoventrally, 0.3 mm. laterally, with minute, irregular transverse ridges in the central unthickened portion of each face, the periphery of the pericarp at least somewhat corky-thickened, glabrous, marginal keel obscure, the style and the achene beak almost lacking, 0.1 mm. long, not recurved.

Borders of lakes and marsh-land at low elevations; Europe; naturalized about Seattle and the islands of Puget Sound, Washington, and at Portland, Oregon; native or naturalized from Michigan and Missouri to Rivere du Loup, Quebec, and York Harbor, Maine, and southward to North Carolina and the Louisiana Delta Region. Mostly coastal in New England. Hardwood, river bottom, and southeastern pine forests. June to September.

Type collection: "Habitat ad Europae fossas & paludes."

60A. RANUNCULUS SCELERATUS var. MULTIFIDUS Nutt. in Torr. & Gray, Fl. N. Am. **1**: 19. 1838. *R. eremogenes* Greene, Erythea **4**: 121. 1896. *R. eremogenes* var. *degener* Greene, Pittonia **4**: 144. 1900. *R. eremogenes* var. *pilosulus* Greene, Pl. Baker, **3**: 2. 1901. *R. eremogenes* var. *pubescens* Lunell. Bull. Leeds Harb. (2): 6. 1908. *R. sceleratus* var. *eremogenes* Garrett, Spring Fl. Wasatch Reg. 25. 1911.

Radical leaves usually with the primary parts or divisions again deeply parted or divided; pericarp smooth except for a circle of minute "pin prick" depressions on each face at the inner margin of the peripheral thickened zone; otherwise like the typical species.

Borders of lakes, ponds, and streams at 1,000–2,100 meters elevation; Alaska (Matanuska) and the Yukon to the Great Basin and the Great Plains as far south as California (northeastern Siskiyou County and Modoc County), Nevada, and Colorado; Sacaton, Arizona, and Rio Arriba and Grant Counties, New Mexico; Minnesota. Best-developed in the northern desert and the plains grassland. May to August.

Type collections: (1) Var. *multifidus*, "Ponds of the Platte, Nuttall." (2) *R. eremogenes*, "Plant of wet springy places and margins of pools in the West American desert regions, through the Great Basin, and to southeastern Oregon and northwestern British America; the American counter-

part of the Old World *R. sceleratus,* to which it has been erroneously referred, being the *R. sceleratus,* var. *multifidus* of Nuttall. . . . Though first detected by Nuttall on the plains of the Platte, the most luxuriant specimens seen by me are from the region of the muddy lakes that lie along the northwestern border of the Great Basin in northeastern California and adjacent Oregon. Smaller specimens, with leaves more finely divided were collected beyond the British boundary, on Milk River Ridge, by Mr. Macoun, in 1895 (n. 10036). . . .'' Apparently *R. eremogenes* was intended as a nomen novum for *R. sceleratus* var. *multifidus,* and that is the interpretation of the writer. Therefore, Nuttall's collection from ''Ponds of the Platte'' is considered to be the TYPE of this species. (3) Var. *degener,* ''Obtained in southern Colorado, in the summer of 1899, by C. F. Baker, perhaps near Pagosa Springs, but the label has been lost.'' *HGr. 2929* and *2930* have C. F. Baker's Pagosa Springs labels and these two sheets are designated as a LECTOTYPE. (4) Var. *pilosulus,* ''In damp places above Gunnison, 17 July, n. 454.'' *C. F. Baker.* The type specimen in the Herbarium Greeneanum is not numbered with the stamp used on the other sheets. The following is quoted from the label, ''in damp spots in bottoms.'' (5) Var. *pubescens,* Leeds, *Lunell.*

60B. RANUNCULUS SCELERATUS var. **longissimus** (Lunell) L. Benson, comb. nov. *R. eremogenes* Greene var. *longissimus* Lunell, Am. Midl. Nat. **1**: 206. 1910.

Glabrous aquatic; stem of the type specimen exceeding 7 dm. above the point of breaking (when collected); leaves all cauline, 1.8–2.3 cm. long, 3–4.3 cm. long, 3-parted, the parts broadly cuneate, again 3–7-lobed or -cleft, the lobes rounded; petioles from submersed nodes up to 35 cm. long, the *stem producing long filiform adventitious roots;* mature fruit unknown.

Running water, Leeds, North Dakota. Summer.

Type collection: ''In running water Leeds [North Dakota], June 27, 1909. Plant rooting from the nodes with long, slender fibers, the lower leaves very long-petioled.'' (Quoted from the label of the TYPE specimen, *Herbarium of the University of Minnesota 257628.*) Collected by *Lunell.*

61. RANUNCULUS GMELINII DC. var. **terrestris** (Ledeb.) L. Benson, comb. nov. *R. Purshii* Richards, Bot. App. Frankl. 1st. Jour. 751. 1823. *R. fistulosus* Pursh ex Torr. Ann. Lyc. N. Y. **2**: 163. 1826, as syn. *R. limosus* Nutt. in Torr. & Gray, Fl. N. Am. **1**: 20. 1838. *R. Purshii* var. *terrestris Ledeb. Fl. Ross.* **1**: 35. 1842. *R. multifidus* Pursh var. *repens* S. Wats. Rept. U. S. Geol. Expl. 40th. Par. **5**: 8. 1871. *R. Purshii* var. *dissectus* Lunell. Bull. Leeds Herb. (2): 6. 1908. *R. Purshii* var. *geranioides* Lunell, Bull. Leeds Herb. (2): 6. 1908. *R. Purshii* var. *radicans* Lunell, Bull. Leeds Herb. (2): 6. 1908. *R. Gmelinii* var. *Purshii* Hara, Rhodora **41**: 386. 1939. *R. Gmelinii* var. *limosus* Hara, Rhodora **41**: 386. 1939.

Glabrous or hairy palustrine or aquatic perennials; stems reclining or floating, rooting adventitiously, 1–4 dm. long and 1.5–3 mm. in diameter, a little branched, commonly 1–4-flowered, fistulous; leaves all cauline and alternate or the basal present and longer-petioled, the blades compactly pentagonal, 1–2 cm. long, 1.5–2.5 cm. broad, very rarely larger, deeply 3-parted or -divided, the divisions 2–3 times forked or sometimes dissected

into ribbon-like divisions, proximally deeply cordate and distally rounded, petioles 1–3 or 4 cm. long, stipular leaf bases 3–6 cm. long; bracts 1–3; petals 5, yellow, circular or obovate, 4–7 mm. long, 3–6 mm. broad, the nectary scale glabrous, its margins prolonged into flaps, the tips of which are usually free from the petal, or the margins joined distally or sometimes as in *R. flabellaris.*

Mud or sometimes shallow water of lakes, stream borders, and marshes up to 1,500 or 2,000 meters elevation; Alaska to 58° 50′ N. Lat. in Keewatin, and to Prince Edward Island and Nova Scotia and southward to Oregon (Klamath Lake), Nevada (Humboldt Wells), Colorado, North Dakota, Minnesota, Michigan, and Maine (Aroostook County); one collection from New Mexico. Northern coniferous, western pine, and northeastern pine forests; plains and prairie grassland. Late May to August.

Type collections: (1) *R. Purshii,* "(W. B.)" "(W) Denotes the wooded country from lat. 54° to 64° north." "(B) Denotes the Barren Grounds from Point Lake to the Arctic Sea." Collected by Richardson. (2) *R. limosus,* "Margins of ponds in the eastern ranges of the Rocky Mountains, Lewis's River, &c. . . . Nutt." (3) var. *terrestris,* "Hab. ad sin: Eschscholtzii in plaga artica Americae boreali-occidentalis! (Chamiss., Eschscholtz, Hook. et Arn.)" (4) Var. *repens,* "Weber Valley, Utah; altitude 5,000 feet; August." *S.* Watson. (5) Var. *dissectus,* "The lobes just as deeply cut [as in var. *schizanthus*], but the segments broader (as in Geranium dissectum L.) The prevalent land form." The specimen collected by Lunell "In margine paludis" at Leeds, Benson County, North Dakota, June 18, 1906 is designated as a LECTOTYPE, *UM 257716.* (6) Var. *geranioides,* "The lobes not as deeply cleft, and the segments still broader than in var. dissectus (the leaf resembles in outline Geranium molle L.)" The specimen collected at Leeds, North Dakota, June 16, 1900, *Lunell 145* is designated as a LECTOTYPE, *UM 257708.* (7) Var. *radicans,* "Creeping, and rooting repeatedly from the nodes. Leaves mostly the form of var. **geranioides.**" The specimen collected in mud on the peninsula of Lake Ibsen, Benson County, North Dakota, July 6, 1908 by Lunell is designated as a LECTOTYPE, *UM 299854. R. Purshii* var. *humifusus* Lunell, Amer. Midl. Nat. **4**: 358. 1916, is a new name for *R. radicans* Regel, Reisen in den Suden von Ost-Sibirien or Pl. Radd. **1**: 44–5. 1861. It is based upon an Old World type, as follows: "Am nördlichen Ufer des Baikel auf sumpsigen Wiesen."

61A. RANUNCULUS GMELINII var. **yukonensis** (Britt.) L. Benson, comb. nov. *R. yukonensis* Britt. Bull. N. Y. Bot. Gard. **2**: 169. 1901. *R. Purshii* subsp. *yukonensis* Porsild, Rhodora **41**: 229. 1939.

Palustrine; stems 0.5–2 dm. long; leaves about 0.8–1 cm. in diameter.

Northern Alaska (reported) and the Yukon District; Marble Mountains, British Columbia (Upper Hat Creek, 3,000 feet, *J. W. & Emily M. Thompson 356, T, B,* July 12, 1938). Northern coniferous forest. Summer.

Type collection: "Mouth of the Bonanza Creek, Dawson, June 18, 1899, R. S. Williams (type)."

61B RANUNCULUS GMELINII var. PROLIFICUS (Fern.) Hara, Rhodora **41**: 386. 1939. *R. multifidus* Pursh. var. *terrestris* A. Gray, Man. Ed. **5**: 41.

1867, not *R. Purshii* var. *terrestris* Ledeb. in 1842 (*R. Gmelinii* var. *terrestris* L. Benson). *R. limoso* × *sceleratus* Greene, Pittonia **2**: 65. 1890. *R. lacustris* Beck & Tracy var. *terrestris* McMillan, Metasp. Minn. Valley 247. 1892. *R. delphinifolius* Torr. var. *terrestris* Farwell, Ann. Rept. Comm. Parks & Boulev. Detroit **11**: 63. 1900. *R. delphinifolius terrestris* Piper, Contr. U. S. Nat. Herb. **11**: 272. 1906. *R. delphinifolius* f. *terrestris* Blake, Rhodora **15**: 164. 1913. *R. Purshii* var. *prolificus* Fern. Rhodora **19**: 135. 1917.

Stems upright; flowers paniculate, about 7–50 per stem; bracts numerous; otherwise like the first variety.

Muddy ground of stream and lake banks at low elevations; in scattered northern localities; Montpelier, Idaho; Belgrade and Bozeman, Montana; Michigan; Magdalen Islands, Quebec. Northern coniferous forest. Summer.

Type collections: (1) Var. terrestris, "Ann Arbor, Michigan, on muddy banks, Miss Clark." Cf. Fern Rhodora **38**: 173. 1936. (2) *R. limoso* × *sceleratus*, "I obtained it in July last, on the muddy shores of Bear Lake, near Montpelier, Idaho." *Greene* in 1889. The TYPE is *HGr. 1171*. (3) Var. *prolificus*, "MAGDALEN ISLANDS: Wet meadow, Grindstone, July 22, 1912, *Fernald, Bartram, Long, & St. John*, no. 7482. (TYPE in Gray Herb.)"

61C. RANUNCULUS GMELINII var. **schizanthus** (Lunell) L. Benson, comb. nov. *R. Purshii* var. *schizanthus* Lunell, Bull. Leeds Herb. (2): 6. 1908. *R. Purshii* var. *polymorphus* Lunell, Bull. Leeds Herb. (2): 6. 1908.

Leaves all more or less deeply dissected, the aerial ones deeply 3-parted or -divided and again 2–3 times parted and cleft, about 3–3.5 cm. in diameter, the submersed ones finely dissected into linear ribbons 1–2 mm. broad, the blades 6–9 cm. in diameter; petals obdeltoid, 6–7 mm. long, 6–7 mm. broad, 2–3-lobed or -cleft, the nectary scales as in *R. flabellaris*.

Sloughs and lake borders at Leeds and Lake Ibsen, Benson County, North Dakota. Known only from the collections of Dr. J. Lunell. Prairie grassland. May and June.

It is possible that this is really a form of *R. flabellaris*. The fruit is unknown.

Type collections: (1) Var. *schizanthus*, "Leaves divided into three lobes, and the lobes finely dissected." No type collection given. The specimen in the Herbarium of the University of Minnesota, collected along sloughs at Leeds, North Dakota, June 1, 1903, *Lunell 448* is designated as a LECTOTYPE, *UM 257701* and *257702*. (2) Var. *polymorphus*, "The emersed leaves present forms 1, 2, and 3 just described. The submersed leaves are capillary multifid (filiform dissected). Stems elongated, sometimes several meters in length." No type collection given. The collection from the Peninsula of Lake Ibsen, North Dakota, May 23, 1908, *Lunell 447* is designated as a LECTOTYPE. It is *UM 257709*, *257710*, and *257711*. The epithet *schizanthus* was applied to a palustrine form and the epithet *polymorphus* to an aquatic form.

62. RANUNCULUS FLABELLARIS Raf. apud Bigel, Amer. Mo. Mag. **3**: 344. March, 1818. *R. multifidus* Pursh, Fl. Amer. Sept. **2**: 736. 1814, not Forsk. in 1775. *R. fluviatilis* Bigel. Fl. Bost. Ed. 1. 139. 1814, not Willd. in 1799.

R. delphinifolius Torr. in A. Eat. Man. Bot. Ed. 2. 395. May or later, 1818, cf. Fern. Rhodora **38**: 171–3. 1936. *R. lacustris* Beck & Tracy in A. Eat. Man. Bot. Ed. 3. 423. 1822. *R. missouriensis* Greene, Erythea **3**: 20. 1895. *R. delphinifolius* f. *submersus* Gluck, Beih. Bot. Cent. **39** (2): 328. 1923. *R. flabellaris* f. *riparius* Fern. Rhodora **38**: 171. 1936.

Shallow water or mud up to 1,500 meters elevation in the West; British Columbia to Eastern Washington and Oregon, the lower Columbia River, to Humboldt, Mendocino, and Modoc Counties, California, to Star Valley, Nevada; and east to Ontario, Maine, and New Jersey; south in the Mississippi Valley to Louisiana. Western pine forest; plains and prairie grassland; harwood forests.

Type collections: (1) *R. multifidus*, "In Upper Louisiana . . . *Bradbury* . . . *v. s. in herb. Bradbury.*" (2) *R. fluviatilis*, "In a pond on Brighton road and elsewhere in deep water." "Within 5 or 10 mi. of Boston." 1812–3. *Bigelow.* (3) *R. flabellaris.* Nomen novum for *R. fluviatilis* Bigel. (4) *R. delphinifolius*, none given. Northern and Middle States. (5) *R. lacustris*, "Very abundant in a small lake east of the village of Lansingburgh," Hudson River, cf. N. Y. Med. & Phys. Jour. **2**: 112. 1823. (6) *R. missouriensis*, "A Missouri species, distributed by Mr. Bush." Apparently the type of this species was not noted by the writer at the Hebaruim Greeneanum in the summer of 1935. (7) *F. submersus*, grown from seed "von Bingen im Staate Washington." (8) *F. terrestris*, grown from seed "von Bingen im Staate Washington." (9) *F. riparius*, Nomen novum for f. *terrestris* above.

DEPARTMENT OF BOTANY, UNIVERSITY OF ARIZONA
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MASS COLLECTIONS: DIERVILLA LONICERA

NORMAN C. FASSETT

In the summers of 1940 and 1941 mass collections were made of *Diervilla Lonicera,* from Minnesota to Maine. Each collection consisted of a twig with one or more pairs of leaves and with flowers or fruits if they were present; these specimens were collected far enough apart to avoid the taking of more than one from a clone. Many of these collections were made possible by a

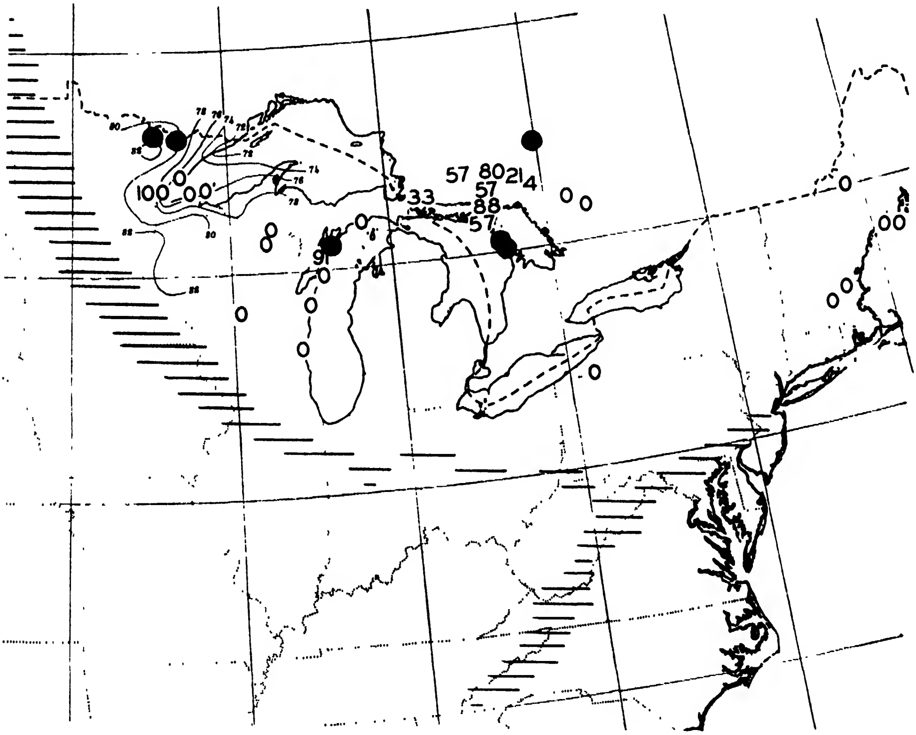

FIG. 1. Lined regions: southern and western limits of *Diervilla Lonicera.* Dots: locations of herbarium specimens of *D. Lonicera* var. *hypomalaca.* Figures: percentage of var. *hypomalaca* in mass collections. Lines connecting italicized figures: average maximum isotherms for July.

grant for travel from the Wisconsin Alumni Research Foundation, and several were contributed by my students, L. H. Shinners and F. W. Stearns. Data concerning distribution of the species in Pennsylvania and in West Virginia, respectively, have been furnished by Dr. J. M. Fogg, Jr., and by Dr. E. L. Core. Figure 1 was prepared from Hall's Outline Map, 801M,

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Examinations of mass collections, totalling some 673 individuals, corroborates in every detail the words of Professor Fernald accompanying his description of var. *hypomalaca*,[1] as follows: "Typical wide-ranging *Diervilla Lonicera* has the leaves glabrous or at most a little setulose along the midrib beneath. Var. *hypomalaca*, known only from a limited area in the range, is striking on account of the dense white pilosity of the lower surfaces of the leaves." There are further details concerning these plants which cannot be studied from conventional herbarium specimens, but upon which light is shed by mass collections.

Pubescence on different parts of the plant acts in various ways. The calyx-lobes and leaf-margins are ciliate, and the ciliation appears to be uni-

TABLE 1

Number of each leaf-type in mass collections of D. Lonicera

	Typical	Intermediate	Var. *hypomalaca*
MINNESOTA: Duluth, Sept. 5, 1940, no. 21417[a]	0	0	10
Two Harbors, Sept. 5, 1940, no. 21257	22	0	0
WISCONSIN: Ravine west of Herbster, Aug. 26, 1940, *L. H. Shinners*, no. 3449	31	0	0
Road to Redcliff Indian Reservation, Bayfield County, Aug. 25, 1940, *Shinners*, no. 3450	11	0	0
Sandy loam, Found Lake, Saynor, June 27, 1941, *F. W. Stearns*, no. 763	39	0	0
Outwash soil, Stormy Camp, on Wisconsin River east of Woodruff, June 27, 1941, *Stearns*, no. 777	10	0	0
Saddle Mound, south of Pray, Aug. 23, 1940, *Shinners*, no. 2713	16	0	0
Shore of Lake Michigan, Peninsula State Park, Fish Creek, Sept. 28, 1940, no. 21258	2	1	19
Limestone beach, Baileys Harbor, July 13, 1940, *Shinners*, no. 2196	35	0	0
Sand dunes, Whitefish Bay, Door County, July 22, 1940, *Shinners*, no. 3447	15	0	0
Sand dunes, Point Beach State Forest, Two Rivers, July 19, 1940, *Shinners* no. 2448	10	0	0
Top of ravine, Doctors Park, north of Fox Point, Milwaukee, Sept. 22, 1940, *Shinners*, no. 3444.	10	0	0
Bluffs north of Klode Park, Milwaukee, Sept. 22, 1940, *Shinners*, no. 3445	2	0	0
Milwaukee River bank north of Locust St., Milwaukee, Sept. 17, 1940, *Shinners*, no. 3446	3	0	0
MICHIGAN: Wooded dunes, Manistique, July 12, 1941, no. 21259	3	0	0
Wooded dunes, Point Seul Choix, July 12, 1941, no. 21260	8	0	0
ONTARIO: Moist woods at foot of bluff, Garden River, July 13, 1941, no. 21261	12	2	4

[a] When a number is given without collector's name the writer was the collector.

[1] Rhodora 42: 144. 1940.

TABLE 1 (*Continued*)

Number of each leaf-type in mass collections of D. Lonicera

	Typical	Inter-mediate	Var. *hypo-malaca*
Along road on rocks, woods, etc., from Little Current to West Bay, Manitoulin Island, July 14, 1941, no. 21262	20	2	24
Pine woods, etc., Great Cloche Island, 2 miles north of Little Current, July 14, 1941, no. 21263	3	0	22
Woods and roadside, 3 miles north of Willisville, July 13, 1941, no. 21264	9	0	12
Granite knob south of Espanola, July 14, 1941, no. 21265	12	3	13
Granite knob east of McKerrow, July 14, 1941, no. 21266	4	2	14
Granite knob at Simon Lake Park, 6 miles west of Copper Cliff, July 14, 1941, no. 21267	19	0	5
Granite knob east of Sudbury, July 15, 1941, no. 21268	27	0	1
Granite knob between North Bay and Callender, July 15, 1941, no. 21269	36	0	0
Hillside and ledges 19 miles east of Mattawa, July 15, 1941, no. 21270	27	0	0
Woods and roadside, Rockland, July 16, 1941, no. 21271	3	0	0
NEW YORK: Wooded roadside, Jones Mt., Steamburg, June 22, 1940, no. 21272	60	0	0
MASSACHUSETTS: Exposed rocks at summit of Mt. Wachusett, Princeton, Aug. 1, 1941, no. 21273	21	0	0
NEW HAMPSHIRE: Rocky roadsides, Londonderry, Aug. 3, 1941, no. 21274	9	0	0
MAINE: Dallas Plantation, north of Rangeley, July 24, 1940, no. 21275	9	0	0
Woods, Southport, July 24, 1941, no. 21276	20	0	0
Exposed ledges, east side of Monhegan Island, Aug. 22, 1941, no. 21277	31	0	0
Totals:	539	10	114

form throughout the species. On some leaves the midribs (and veins) are setulose below, as is mentioned in Professor Fernald's paper. The number of plants showing this character varies irregularly in mass collections, and close inspection of a number of herbarium specimens shows that some leaves on a plant may be completely glabrous and others on the same plant have setulose midribs.

In table 1 the collections are listed, with the number of individuals of each type represented. From the figures in this table have been computed the percentages of var. *hypomalaca* at each station (including as the variety the intermediates and plants with appressed hairs on the leaves), and these percentages have been placed on figure 1. The variety appears in three areas: one is from Bruce Peninsula, Ontario, northward, a second is in Door County, Wisconsin, and a third is in northeastern Minnesota. It is quite possible that further collections from the Upper Peninsula of Michigan and

from the country north of Lake Superior would show these three areas actually to be confluent. With our present limited data, however, we must for the present discuss these as three separate areas.

In Ontario, from Great Cloche Island (88 per cent on figure 1) to McKerrow (80 per cent), appears a center where each colony of *Diervilla Lonicera* consists of a large majority of var. *hypomalaca;* to the east and west, and possibly to the south (data are lacking to the north), the percentages of velvety-leaved plants in each colony drops progressively. To the east, in particular, is exhibited a well-marked cline.[2]

In Door County, Wisconsin, var. *hypomalaca* appears in but one mass collection, where it makes up 91 per cent, comprising 20 individuals of 22. That these 20 individuals do not represent merely one extensive clone is shown by slight individual variations in nature and density of pilosity and in color of leaves. This collection is from a limestone beach on the Green Bay side of the peninsula, which is the region of origin of one of the original sheets cited with the description of the variety, and of the only sheet in the Herbarium of the University of Wisconsin. Two other mass collections from Door County, comprising 50 individuals, include none of the variety. These two collections are from the Lake Michigan side of the peninsula; one is on sand, and the other on a limestone beach. Soils differences are not, then, responsible for the differences between the two sides of the peninsula. Var. *hypomalaca* occurs only in the northern part of the range of *D. Lonicera*, but the west side of Door County, where it is found, is warmer and less humid[3] than the east side, where it has not been found.

In Minnesota, as in the region just discussed, the occurrence of var. *hypomalaca* does not appear to be related to low temperature. A mass collec-lection from the heights above Duluth is made up entirely of the variety, and from there it occurs northward; two herbarium specimens have been taken near the Canadian border.[4] The region north of Duluth is warmer than the Lake Superior shore, as is shown by the July average maximum isotherms on figure 1.[5] The boreal plants of this region ordinarily occur, not northward from Duluth, but northeastward along the Lake Superior shore, but the velvety-leaved variety is absent from the one mass collection from Two Harbors and is not represented by herbarium specimens.

Pilosity of lower leaf-surface in *Diervilla Lonicera* is definitely not a response to exposure. The three collections from Lake Huron northward (57

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[2] A cline is "a chain or gradient of differences arranged in a definite direction." Goldschmidt, R. B., *The Material Basis of Evolution,* p. 65.

[3] Cf. Fassett, Ann. Mo. Bot. Gard **28**: 337–338. 1941.

[4] Loaned from the Herbarium of the University of Minnesota by Professor C. O. Rosendahl.

[5] Data from *Climatic summary of the United States,* Section 44—Northern Minnesota, p. 18.

per cent, 88 per cent, and 57 per cent, respectively, on figure 1) were each made partly in the open and partly in the woods, and it was observed in the field that there was no correlation of exposure and pilosity. From near Espanola to Callender, Ontario, all collections were made in the same habitat, namely the heavily glaciated, almost bare, granite knobs, and these collections show a well-marked cline (80 per cent, 21 per cent, 4 per cent, 0 per cent). That the percentage of occurrence of the variety is not affected by exposure is further indicated by the collections from the heavily glaciated summit of Mt. Wachusett, Massachusetts, and from the sea-cliffs of Monhegan Island, Maine; both are similar to the granite knobs of Ontario but neither has any pilose plants. Var. *hypomalaca* is strictly limited to a geographic area without respect to any observed ecological factor.

The varieties of *D. Lonicera* cross a conspicuous lithological boundary. Manitoulin Island (57 per cent on figure 1) and Great Cloche Island (88 per cent on figure 1) are composed of Silurian and Ordovician limestones, but a few miles north of Great Cloche Island there is an abrupt change, the limestone giving way to granites, quartzites, etc., of Huronian and Pre-Huronian age. Neither the abundance of the plant nor the percentages of its varieties seem to be affected by this change in rock type.

The distribution of *D. Lonicera* var. *hypomalaca*, not being related in any obvious way to ecological factors, must be the result of its history. Perhaps it represents a mutation which has infiltrated the species from a single center of origin; perhaps it is a pubescent race, once occupying a region north of the Great Lakes, which has been submerged by the spread of the glabrous race.

Goldschmidt[6] writes, "One of the most remarkable features of these clines of subspecific characters is that they frequently occur in the form of a continuous fluctuation of characters, so that two distinct forms are connected by a continuous series of intermediate conditions." Such gradations between varieties and the typical forms of their species may be readily recalled, but such is not the case here. Of 673 individuals, including 134 of var. *hypomalaca*, but 10 have pubescence so sparse or inconspicuous that they may be considered intermediate, and these are all in collections where well-developed individuals of the variety are in the majority. The gradation consists less of the development of intermediate individuals than in the varying numbers, at each locality, of definitely pilose individuals as compared to the number of definitely glabrous individuals.

A few plants from the Manitoulin Island collection, where 57 per cent of the individuals are var. *hypomalaca*, have the lower leaf-surfaces without the ordinary velvety pilosity of the variety, but rather with copious long straight appressed hairs. These plants are treated as var. *hypomalaca*.

[6] L.c., p. 66.

A strange individual was collected in the woods a few miles south of Mt. Wachusett, Massachusetts. Two stems arise from opposite sides of the rootstock; one is normal and the other has alternate leaves.

SUGGESTIONS

Solution to these problems in taxonomy and phytogeography can be approached only by the taking of mass collections throughout a large area. In many regions phases of these problems can be studied by local botanists. Here, perhaps, is the justification for this publication of results based on fragmentary evidence.

In the Arrowhead Country of Minnesota, and in adjacent Ontario, mass collections should affirm or disprove the suggestion that var. *hypomalaca* is indifferent to temperature. Is it really absent from the north shore of Lake Superior? Would collections from north of the lake show the range of the variety to be continuous from Duluth to Sault Ste. Marie and eastward? There are but 2 mass collections, comprising 8 individuals, between Sault Ste. Marie and Door County, Wisconsin; is the variety actually isolated in Door County? Would a series of mass collections from the tip of Bruce Peninsula southward show a cline? And what of the species as it occurs north of the regions represented by these mass collections? A botanist driving north from North Bay would have opportunity to determine whether the variety becomes less abundant again northward, or if it finally replaces entirely the glabrous plant.

SUMMARY

Diervilla Lonicera shows 3 types of pubescence, each of which has a different manner of occurrence. (1) Plants with densely pilose lower leaf-surfaces occur in 3 regions, one north of Lake Huron, another west of Lake Michigan, and a third in northern Minnesota. They are nearly always accompanied by other individuals with glabrous leaves, and show clines to the south, east and west from a center of high percentage just north of Lake Huron. Intermediates are rare. All these facts seem to justify the treatment of the pilose-leaved phase of *D. Lonicera* as var. *hypomalaca* Fernald. The clines show no relation to exposure, rock types, or local differences in temperature, apparently representing a purely geographic concentration. Var. *hypomalaca* may be a "submerged species," or may be the result of the infiltration of the species by a pilose mutation. (2) The occurrence of leaves with midrib and/or veins setulose below is sporadic throughout the range of the species, and such leaves may even occur on the same plant with perfectly glabrous leaves. (3) Ciliation of leaves and calyx-lobes is essentially uniform throughout the species.

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INDEX TO AMERICAN BOTANICAL LITERATURE

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Papers that relate exclusively to bacteriology, forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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PLANT TAXONOMY AND FLORISTICS
(exclusive of fungi)

(See also under Genetics: **Hubricht & Anderson**)

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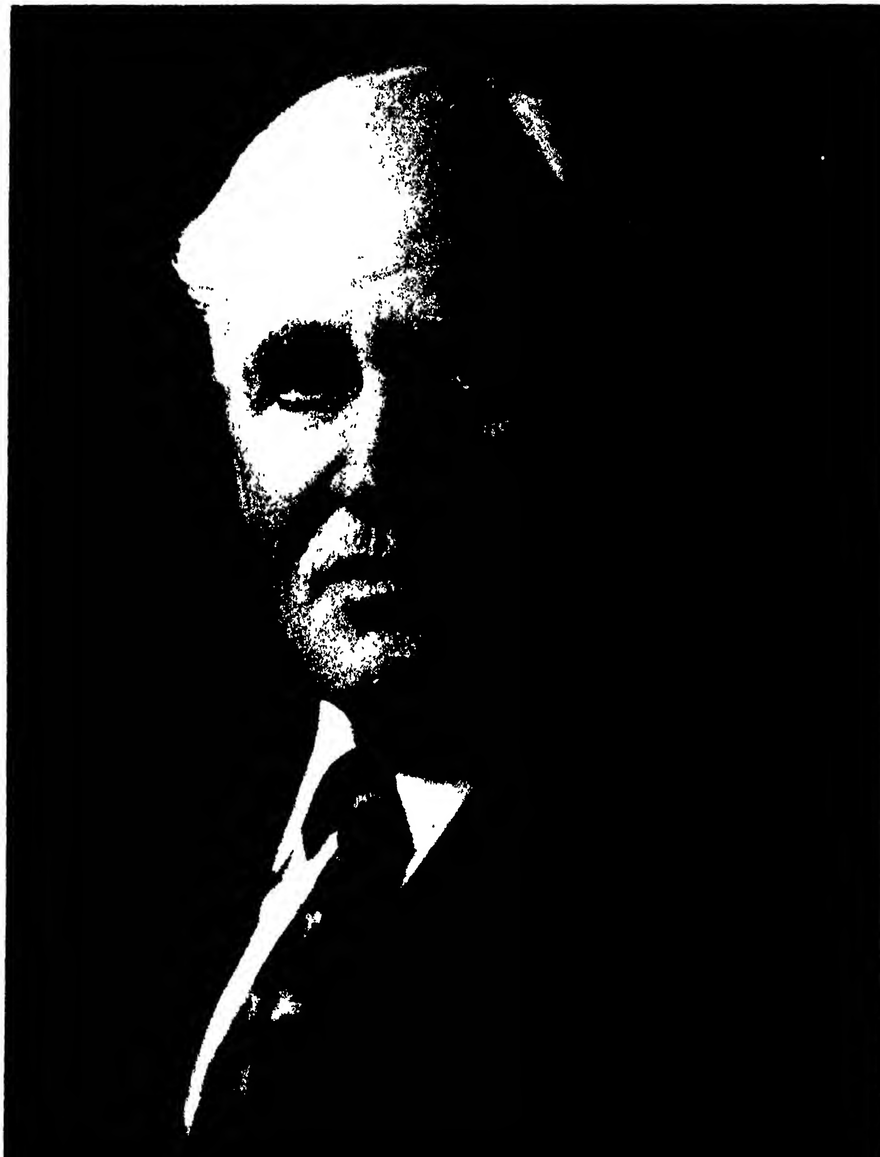

ROBERT ALMER HARPER

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Robert Almer Harper was born at Le Claire, Iowa, on January 21, 1862. He was the son of the Reverend Almer Harper and Eunice [Thompson] Harper. He received the degree of Bachelor of Arts from Oberlin College in 1886, and immediately began his career as a teacher. For two years (1886–1888) he taught Latin and Greek at Gates College in Nebraska. From 1889 to 1891 he was instructor in science at Lake Forest Academy, Lake Forest, Illinois. In 1891 he returned to Oberlin for the degree of Master of Arts, and became professor of botany and geology at Lake Forest College. He continued the study of botany at Johns Hopkins University and at the University of Bonn; in 1896 the latter institution conferred on him the degree of Doctor of Philosophy. In 1898 he became Professor of Botany in the University of Wisconsin, where he remained for thirteen years. He came to Columbia University in 1911 as Torrey Professor of Botany. He remained at Columbia until 1930, when he retired with the title of Professor Emeritus.

Professor Harper was elected to the Torrey Botanical Club in 1911, and from that time until his retirement was one of its most active members. He was a member of the finance committee in 1912 and 1913 and from 1917 to 1932, and was also a member of the budget committee during the latter period. He was president of the Club during 1914, 1915, and 1916. He served also on the local flora committee and as a member of the council. He attended almost every meeting of the Club, and was ever ready to participate actively in the discussions. During his early years at Columbia he also showed great interest in the field trips and often organized special excursions for groups of students in the department of botany. He bought a large Panhard automobile in which he carried students to the Pine Barrens of New Jersey, to Cold Spring Harbor, Long Island, and to many other places in the Torrey Club area. Those who accompanied him on these outings carried away the most pleasant memories.

The researches for which Professor Harper is best known are his classic studies in the morphology and cytology of the slime molds and the ascomycetes. The beautiful figures with which his papers were illustrated have long served as models for later workers. A number of his later papers dealt with the evolution of cell types and problems of morphogenesis as elucidated by *Pediastrum*, *Hydrodictyon*, and *Dictyostelium*.

His interests have never been confined to his own specialities; few men have had a broader knowledge of botany in all its phases. This is reflected in the thorough training of his students, many of whom have attained eminence. His keen and active mind earned the respect and admiration of those who worked under him and made him successful as a teacher.

It is with pleasure and satisfaction that, as the Torrey Club celebrates the Seventy-fifth Anniversary of its founding, we congratulate Professor Harper on his eighty years of life.

The portrait of Doctor Harper is published with the assistance of the Lucien M. Underwood Memorial Fund.

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THE ROOTING OF FLOWERS IN STERILE CULTURE[1]

CARL D. LA RUE

INTRODUCTION

The question of the ability of various organs of the plant to regenerate roots and buds has been of theoretical and practical interest for ages. Stem cuttings have been used to propagate plants for periods unknown, but certainly very long. The recent discovery of the role of growth hormones in root production has extended greatly the possibilities of rooting cuttings with promising practical results. The author (13) has shown recently that wounding also stimulates root formation, and this may be a further aid in propagation.

The production of new plants from leaves is current practice for *Begonia, Saintpaulia,* and other species, and Kupfer (7) showed that the leaves of 61 species could be rooted. She also demonstrated the power of root formation in modified leaves such as leaf-thorns, phyllodes, and juvenile leaves. Propagation from bud scales has been described by several writers and has long been practiced by bulb-growers. Kupfer studied this process in the onion and several investigators have described it from work on bulbs of other species. The author (8) has rooted cotyledons of more than 40 species and Stingl (16) was able to root leaves of more than 100 species. In the past thirty years a series of papers has appeared describing the regeneration of leaves of species too numerous to list here.

Regeneration of buds on roots has long been known and such plants as horseradish probably have been propagated by root cuttings since their earliest cultivation. Although much remains to be learned concerning the factors which lead to bud formation on roots, regeneration in roots has received attention from Vöchting (17), Goebel (2), Kupfer (7), Beals (1), Graham and Stewart (5), Naylor (14), and numerous others.

The flower has been regarded usually as an organ specialized for seed production and possessed of little capacity for proliferation. The inflorescence also is rarely involved in vegetative reproduction though some plants regularly produce bulblets or offshoots of some kind on their inflorescences (*Ananas sativa, Anthophytum pictum, Furcraea pubescens*). This may explain why regeneration of root, stem, and leaf has received much attention from botanists, whereas the fruit and the flower as organs of vegetative

[1] Papers from the Department of Botany, University of Michigan, No. 727. Grateful acknowledgement is made to the Division of Biology, Harvard University, for facilities granted the author during the progress of this study.

propagation have received relatively little notice. Goebel (4) cites reports of rooting of fruits in the Cactaceae where this phenomenon appears to be of rather common occurrence. He also mentions Baillon's results in rooting ovaries of *Jussiaea* and Martius' statement that the very large unripe fruits of *Lecythis* produce roots and shoots when planted in the earth.

Kupfer (7) cites an observation by Carrière that roots were formed on a capsule of *Lilium speciosum,* and from her own experiments describes the successful rooting of half-grown pods of the bush bean and of the lima bean.

Several cases of the rooting of peduncles and inflorescences are known. According to Kupfer (7), Beinling observed this in *Echeveria,* Sorauer in *Primula,* and Hansen in *Achimenes.* Goebel (2) reported the process in *Ajuga, Klugea, Naegelia,* and *Tussilago.* Kupfer herself rooted inflorescences of *Dudleya californica* and peduncles of *Bryophyllum crenatum* and *Ruellia rosea.* Bud formation appears to be less common than root formation on floral axes but it is reported by Goebel (3) for *Tussilago farfara* and by Kupfer for *Dudleya californica.* Graham and Stewart (6) were able to propagate new plants from pieces of the head of broccoli.

The author (10) has investigated the capacity for cell proliferation in various tissues of many plants with a view to determining which of these contained what Erwin F. Smith (15) called totipotent cells. As a result of his observations he made a tentative classification dividing tissues, organs, and species of plants into two groups; those highly specialized and those which are totipotent or capable of vegetative proliferation and propagation. The same idea appears to be widespread among botanists, but the results gained by the application of growth substances show that although such a classification may have some value in considering plants under normal growth conditions, the specialization of tissues and loss of regenerative capacity is more apparent than real.

Stem internodes, in which morphological category the pedicels of flowers fall, have been regarded by the author as specialized structures, and flowers themselves were thought to be too closely connected with seed production to be capable of formation of roots and buds. The technique of growing embryos and plant fragments in culture (9) seemed to offer a means of testing the vegetative activity of flowers under optimum conditions. The present study is an attempt to discover to what extent flowers and floral organs are capable of regeneration.

MATERIALS AND METHODS

Flowers of 12 species of the Monocotyledoneae belonging to 9 genera and 3 different families were placed in culture on agar. Of the Dicotyledoneae 80 species of 74 genera and 34 families were cultured. The flowers were usually cultured just after opening or just before opening. Young buds of several species were cultured but nearly all of these failed to form roots.

The flowers were sterilized with bromine water reduced to one-fourth concentration (11). They were placed in closed containers, immersed and shaken up in the liquid for varying lengths of time. Five minutes proved a fairly satisfactory length of time for many species. There was always a question of the tolerance of the structures to the bromine solution. More tolerant species were given longer periods of sterilization. In most of the species it was impossible to insure a high degree of sterilization without killing the flowers, and it was necessary to become reconciled to the loss of most of the flowers by the growth of bacteria and fungi. Probably all or nearly all these microorganisms were saprophytes which killed the flowers by growing over them and smothering them. The results indicate that all healthy plants carry on their surfaces a very large number of species of bacteria and fungi. Their removal from, or destruction on, the complexly folded and often hairy surfaces of flowers constituted the most difficult part of this work. Hundreds of contaminated cultures had to be made to secure a relatively small number of cultures either sterile or so little contaminated as to allow the flowers to continue life in culture.

White's solution (19) of inorganic salts with the addition of 2 per cent sucrose but without yeast extract was used as a nutrient. Liquid cultures did not give good results, and 0.8 per cent agar was added to the nutrient medium to solidify it. No growth hormone was added to the medium used for the early cultures, but later indole-3-acetic acid was added, one part per hundred thousand. After sterilization the flowers were pressed gently on the slanted agar surface.

All cultures were made in glass bottles of the type known as "½ oz. square packers." These bottles were fitted with screw tops of bakelite which endure repeated sterilization without damage. When these caps are screwed on firmly they prevent loss of water from the agar and they are also effective in preventing the entrance of contaminating spores.

All the cultures were kept at ordinary laboratory temperatures and exposed to light from a north window.

OBSERVATIONS

Root Formation. Visible roots were produced on flowers of 3 genera of 2 families of the Monocotyledoneae and of 25 species of 22 genera and 15 families of the Dicotyledoneae. The species which formed roots and the lengths of time required for rooting are given in table 1.

Extent of Root Development. Root development was limited in the greater number of species. Although a number of roots were produced on each specimen of many of the flowers (figs. 2, 5), they rarely exceeded 1 cm. in length. Extensive and apparently normal root systems were formed on flowers of *Echeveria eximia, Nemesia strumosa,* and *Kalanchoë globulifera.*

Effect of Growth Hormones. It is apparent from table 1 that numerous species can form roots on flowers in culture without the addition of growth hormones. An equal number of species produced roots on flowers in culture with the addition of 1 part per 100,000 of indole-acetic acid to the nutrient medium. Apparently the time needed for rooting is shorter when indole-acetic acid is added. Comparative data are shown for only two species of *Kalanchoë* and the number of individuals was too small to give anything more than a suggestion of a possible effect.

TABLE 1

Time required for the rooting of flowers in sterile cultures

Species	Days required for rooting	
	On nutrient agar without hormone	On nutrient agar with indole-3-acetic acid 1/100,000
Asclepias syriaca	...	22
Begonia scharffiana	45	...
Begonia schmidtiana	...	9
Calendula officinalis	27	...
Caltha palustris	...	13
Echeveria eximia	...	25
Epilobium angustifolium	...	12
Euphorbia splendens	...	12
Forsythia suspensa	...	91
Freesia refracta	...	14
Helianthus annuus	24	...
Impatiens balsamina	41	...
Kalanchoë globulifera	33	10
Kalanchoë sp.	15	8
Nemesia strumosa	19	...
Nerium oleander	...	14
Radicula aquatica	12	...
Radicula armoracia	...	14
Reinwardtia indica	...	71
Saxifraga sarmentosa	15	...
Sedum spectabile	...	25
Swainsonia galegifolia	36	...
Taraxacum officinale	26	...
Tradescantia paludosa	10	...
Viburnum carlesii	...	174
Vinca rosea	36	...
Zebrina pendula	34	14

Considering the widespread interest in the effect of plant-growth hormones on the production of adventitious roots of a great variety of plants, it is desirable to know the efficacy of these substances in the production of roots on flowers. Several attempts were made to secure adequate data but these have failed completely because, either the sterilization was so severe as to kill all the flowers, or was so ineffective as to allow contamination by microorganisms to spoil the experiments. Since it appears that this is a special problem in itself, it has been given up for the present.

Position of Roots. Roots have usually appeared on the pedicels of the flowers but in several species where the flowers were removed from their pedicels roots grew out from the bases of the ovaries. This was true of *Kalanchoë globulifera, Impatiens balsamina, Freesia refracta,* and *Tradescantia paludosa* (fig. 1). In *Helianthus annuus, Calendula officinalis,* and *Taraxacum officinale* a single root always grew out from the vascular bundle at the base of the ovary.

Polarity. Complete polarity was shown by all the specimens. Roots developed at the bases of the pedicels (figs. 1, 2) or if pedicels were lacking the roots grew out from the bases of the ovaries. In relatively long pieces of pedicel of *Begonia* (fig. 5) roots formed along the whole length of this organ.

Longevity of Tissues. A surprising result of culturing flowers and pieces thereof is the great longevity of the tissues, an account of which has been published (12). Whole flowers, pieces of petal, 0.5 mm. × 1 mm., and stamen hairs of *Tradescantia paludosa* have remained alive for a whole year. Flowers of many species have survived for months although they usually become considerably discolored. In spite of a widespread and erroneous account in the public press to that effect, "cut flowers" have not been kept "perfectly fresh and beautiful" in these cultures.

Abscission of Parts. In general the parts of flowers remained intact in culture but abscission of parts appeared in certain species. The pedicels abscised from *Gasteria* after 11 days. Corollas of full-grown flowers of *Nerium oleander* abscised after 13 days and those of buds only one-fourth grown separated from the flowers after 18 days. Petals of *Jasminum fruticosum* abscised after months in culture. In *Helianthus* and *Calendula* the the ovaries separated from many of the flowers after a period of three months in culture.

Bud Formation. Bud development was seen in only two species, *Nemesia strumosa* and *Kalanchoë globulifera.* In *Nemesia* the buds arose at the bases of the ovaries and were suspected of being preformed rather than regenerated buds. In *Kalanchoë,* however, the buds developed on the cut ends of pedicels and undoubtedly arose by true regeneration.

Propagation of Plants from Flowers. The new buds of *Nemesia* developed on flowers with extensive root systems. They grew out rapidly and formed normal but frail plants which grew to a height of about 6 centimeters. They were transplanted to earth and kept under bell jars to prevent wilting; but they all soon succumbed to damping-off fungi. Aside from their small size they appeared different in no way from plants grown from stem cuttings.

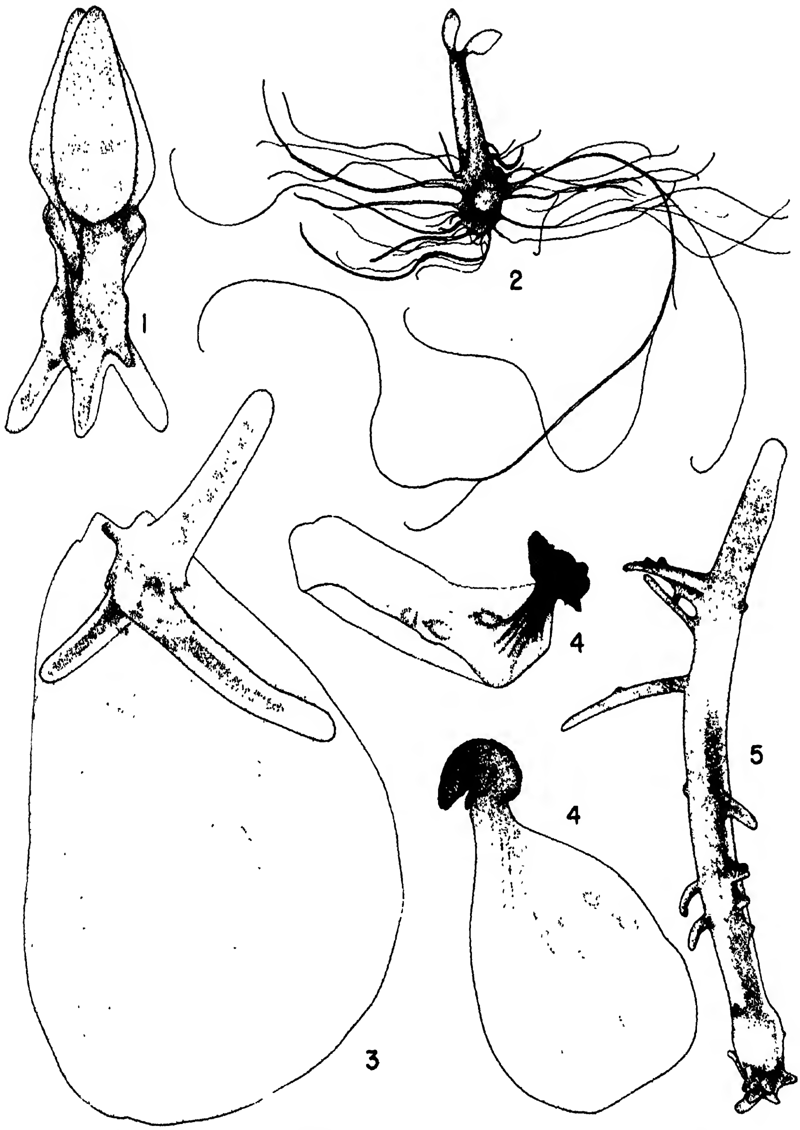

FIG. 1. Rooted bud of *Tradescantia paludosa*. FIG. 2. Flower of *Kalanchoë globulifera* with bulbil at base and numerous roots. FIG. 3. Petal of *Epilobium angustifolium* with three roots. FIG. 4. Petals of *Epilobium angustifolium* with roots. FIG. 5. Pedicel of *Begonia scharffiana* with numerous roots. (Figures drawn by Thomas Cobbe from photographs.)

The *Kalanchoë* buds were exceedingly small and grew slowly. Good root systems developed (fig. 2), and the tiny plants were transferred to earth. The flower buds withered away, tiny leaves appeared, and the plants showed every sign of being able to continue growth when they were all lost through oversight in watering the greenhouse.

The dependence of root upon shoot was seen in the superior development of roots on the flowers which had developed leaf buds. However, extensive root systems were developed on flowers of *Kalanchoë globulifera* and *Echeveria eximia* which had not produced buds.

Intumescences. The sepals of tomato invariably produced intumescences over their entire upper surfaces in culture. Similar outgrowths were frequently seen on ovaries of *Freesia refracta, Passiflora biflora, Kalanchoë globulifera, Rheinwardtia indica,* and *Impatiens balsamina.*

Callus Formation. Callus formation was infrequent on flowers. It was seen on petals and stamens of *Hibiscus rosa-sinensis,* on bases of detached involucral bracts of *Calendula officinalis,* and rarely on the cut bases of pedicels of *Kalanchoë globulifera.*

Bulbil Formation. In numerous instances the bases of pedicels became swollen to three or four times their original diameter so that a spherical bulbil was formed at the base of each. This occurred without reference to root formation although these structures were most frequent in *Kalanchoë globulifera* (fig. 2) and *Echeveria eximia,* species which readily produced roots.

Growth of Floral Organs in Culture. Immature flower-buds in culture never grew to full size and in general it appeared that practically no growth took place in any of the flowers except in an irregular fashion. Such irregular growth was common and manifested itself in the curling of petals in *Freesia refracta, Epilobium angustifolium, Tulipa gesneriana, Hibiscus rosa-sinensis, Althaea rosea,* and *Tradescantia paludosa.* Twisting of stamens occurred in *Hibiscus rosa-sinensis* and *Althaea rosea.* Twisting and curling of sepals took place in *Tulipa gesneriana* and *Tradescantia paludosa.*

Fruit Development. Fruits developed from flowers of several species. Tomato fruits increased in size fivefold, and fruits of *Kalanchoë rotundifolia* developed to practically full size, though the seeds inside them contained no embryos. *Forsythia suspensa* fruits, whether rooted or not, remained alive in culture a full year and reached almost their maximum size. *Caltha* fruits grew to about one-half normal size and burst open, exposing undeveloped ovules.

Rooting and Development of Parts of Flowers. Flowers of a number of species were dissected and the parts put on agar. Although many of these survived in culture for months the only result from most of them was a noticeable amount of twisting and curling especially frequent in petals and stamens.

However, detached ovaries of *Asclepias syriaca* and of *Radicula aquatica* formed roots at their bases and the very small petals of *Epilobium angustifolium* formed roots at their bases within two weeks on nutrient agar containing indole-3-acetic acid. Usually a single root was formed (fig. 4), but several produced two roots, and one petal grew three prominent roots (fig. 3). This is remarkable when one considers the fragility, small size, and short life of these structures on the plant. None of these roots became more than 2 cm. long.

Ovules of several species were placed in culture on agar and in liquid media. Those of *Erythronium americanum* increased noticeably, producing irregular and twisted growth of their seed coats. Most of them quadrupled their original size. Snapdragon ovules produced an extensive mass of callus-like tissue all over their surfaces, apparently much more voluminous than White (18) secured in his cultures of the same objects. Although these ovules remained alive for months, growth in them did not continue.

DISCUSSION

Apparently the ability of plants to form roots on their flowers is widespread and from the results secured it is difficult to see that any one family is especially favored in this respect. It is possible that root primordia were formed on other flowers but did not emerge. Sections were not made to determine whether or not this was true.

It does not seem worth while to list the species which have not rooted in culture, for it is not certain that they will not root under proper conditions. The greater number of cultures were ruined by contamination before sufficient time had elapsed for root formation. *Jasminum fruticosum* is a species which appears difficult to root since numerous cultures of this remained sterile for months without producing roots.

The tomato also was tried repeatedly without result though the plant as a whole is regenerative to a high degree.

The absence of the Cactaceae in the list of flowers rooted may seem curious in view of the fact that cactus fruits of several species are known to root naturally in the open. A number of species of several genera were tried in culture, but could never be sterilized. Dr. Elzada Clover of the Department of Botany of the University of Michigan reports success in rooting flowers of a number of species of Cactaceae in sand.

The inability of the flower to produce roots is apparent rather than real,

and the reasons this inability previously appeared to exist are: 1, that flowers are seldom found in contact with a moist substratum; 2, that they do not survive long enough to enable roots to be formed; and 3, that they are too small to contain a sufficient supply of food to induce regeneration. Fruits and flowers of the Cactaceae often fulfill these requirements, and so do the fruits of *Lecythis* which are very large structures often as much as 25 cm. in diameter.

In culture flowers are under optimum conditions so far as moisture is concerned; the medium supplies an adequate source of food and in some unknown way the life of the cells is prolonged (12) to an extraordinary degree so that time is given for root formation.

SUMMARY

1. Tests for regeneration on nutrient agar were made on flowers of 12 species of the Monocotyledoneae belonging to 9 genera and 3 families, and on 80 species of 74 genera and 34 families of the Dicotyledoneae.

2. Roots were produced on flowers of 3 genera of 2 families of the Monocotyledoneae and of 25 species of 22 genera and 15 families of the Dicotyledoneae.

3. It is concluded that the apparent lack of regeneration in flowers is due to: 1, their short life which does not allow time for root or bud formation; 2, their position which does not allow them contact with moist substrata; and 3, their lack of a sufficient food supply.

4. The conditions needed by flowers for regeneration are supplied by placing them in sterile culture on nutrient agar. In addition to supplying moisture and food the cultural conditions prolong the life of the flowers to an extraordinary degree and allow them time for regeneration.

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SPECIFICITY OF PYRIDOXINE FOR CERATOSTOMELLA ULMI

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In an earlier paper (8) we reported that *Ceratostomella ulmi* had a complete deficiency for pyridoxine. This organism did not grow in a basal mineral-dextrose medium containing asparagine unless pyridoxine was added. How specific is this response? Can the effect of pyridoxine on *C. ulmi* be produced by nearly related compounds?

METHODS AND MATERIALS

An isolation of *C. ulmi* derived from a single conidium was grown at 20° C in 125 ml. Erlenmeyer flasks with 25 ml. quantities of a basal mineral-dextrose containing asparagine.[1] Dry weights were determined by filtering through a Gooch crucible and drying at 100° C. The organism was grown also on sloped agar in test tubes containing 8 ml. of the basal medium solidified with 1.5 per cent purified agar (7). The twelve analogs tested were supplied through the courtesy of Merck and Co. The identification numbers and names of the analogs were as follows:

- No. 1421 Hydrochloride of triacetate of vitamin B_6.
- No. 1422 Hydrochloride of diacetate of vitamin B_6.
- No. 1442 Hydrochloride of β-methyl ether of vitamin B_6.
- No. 1443 Hydrochloride of 2,4,5 trimethyl-3-hydroxypyridine.
- No. 510 Lactone of 2-methyl-3-amino-4-hydroxymethyl-5-carboxypyridine.
- No. 511 Lactone of 2-methyl-3-hydroxy-4-hydroxymethyl-5-carboxypyridine.
- No. 1029 2-methyl-3-amino-4-hydroxymethyl-5-aminomethyl pyridine dihydrochloride.
- No. 1030 2-methyl-3-hydroxy-4-ethoxymethyl-5-hydroxymethyl pyridine hydrochloride.
- No. 1031 2-methyl-3-amino-4-ethoxymethyl-5-aminomethyl pyridine dihydrochloride.
- No. 1032 2-methyl-3-hydroxy-4,5-epoxydimethyl pyridine hydrochloride.
- No. 1797 Hydrochloride of 2,4-dimethyl-3-hydroxy-5-hydroxymethyl pyridine.
- No. 3882 2-ethyl-3-hydroxy-4,5-bis-(hydroxymethyl)-pyridine hydrochloride.

[1] This solution contained per liter 50 g. dextrose, 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4 \cdot 7H_2O$ and 2 g. asparagine plus the following trace elements in p.p.m.: 0.005 B, 0.02 Cu, 0.1 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo and 0.09 Zn.

Pyridoxine and its analogs were added in millimicromoles (mμ moles) per tube or per flask in order that their relative effectiveness could be compared on a molar basis. All glassware was cleaned with chromic-sulfuric acid cleaning mixture. Since cotton batting had been found to contain appreciable amounts of pyridoxine (8) flasks and test tubes were stoppered with washed cotton. This precaution was probably unnecessary as the few strands of cotton which fell in the medium in a flask or test tube plugged with unwashed cotton were not observed to have any effect on the growth of *C. ulmi.* All solutions were sterilized in an autoclave at 15 lbs. pressure for 20 minutes.

EXPERIMENTAL RESULTS

Quantity of Pyridoxine and Growth of C. ulmi. Before the effect of the analogs of pyridoxine could be compared with that of pyridoxine itself, it was necessary to determine the response of *C. ulmi* to different amounts of pyridoxine. Both liquid and agar media were used.

The dry weight produced at 20° C in 25 ml. of the basal liquid medium in 125 ml. Erlenmeyer flasks in a period of 14 or 20 days was approximately the same in the presence of 5, 10, 25, or 50 mμ moles of pyridoxine per culture (table 1). A marked decrease in growth occurred when the quantity

TABLE 1

Effect of pyridoxine on growth of C. ulmi in mineral-dextrose solution containing asparagine

Additions to 25 ml. of basal solution	Dry wt. per culture mg.	
	20 days	14 days
50 mμ moles pyridoxine	25.1	.....
25 mμ moles pyridoxine	24.5	.....
10 mμ moles pyridoxine	27.8	.....
5 mμ moles pyridoxine	26.3	.....
1 mμ mole pyridoxine	15.3	15.2
0.1 mμ mole pyridoxine	7.4	6.2
0.01 mμ mole pyridoxine	.....	0.4
0.001 mμ mole pyridoxine	.....	0.1
None	Trace	Trace
0.3 g. malt extract	119.7	....

of pyridoxine was reduced to 1 mμ mole, but the effect of 0.001 mμ mole was measureable. The benefit from 0.0001 mμ mole could be detected by the eye, but the growth was too small to be weighed. The minimum time for maximum growth was not determined. It appeared to be between 7 and 14 days.

The mucilaginous character of the growth of *C. ulmi* in our medium made filtration through Gooch crucibles a slow and time-consuming process. An attempt was made to determine the growth photometrically. No difficulty was experienced in measuring differences in the growth in the basal medium,

and in the same medium containing 1.0, 0.1, 0.01, 0.001, and even 0.0001 mμ mole of pyridoxine. However, the method was not considered entirely satisfactory because the mixture of mycelium and yeast-like cells which was obtained gave a suspension which was not optically uniform.

On agar slopes of the basal medium plus 1.5 per cent purified agar, little difference was noted in the effect of 5, 10, or 25 mμ moles of pyridoxine per tube. Somewhat slower development occurred with 1 mμ mole, and a marked decrease in the thickness of the mat was found with 0.1 mμ mole. A definite beneficial effect was observed with 0.01 mμ mole, and a slight effect with 0.001 mμ mole (fig. 1). Because the agar cultures were more simply handled

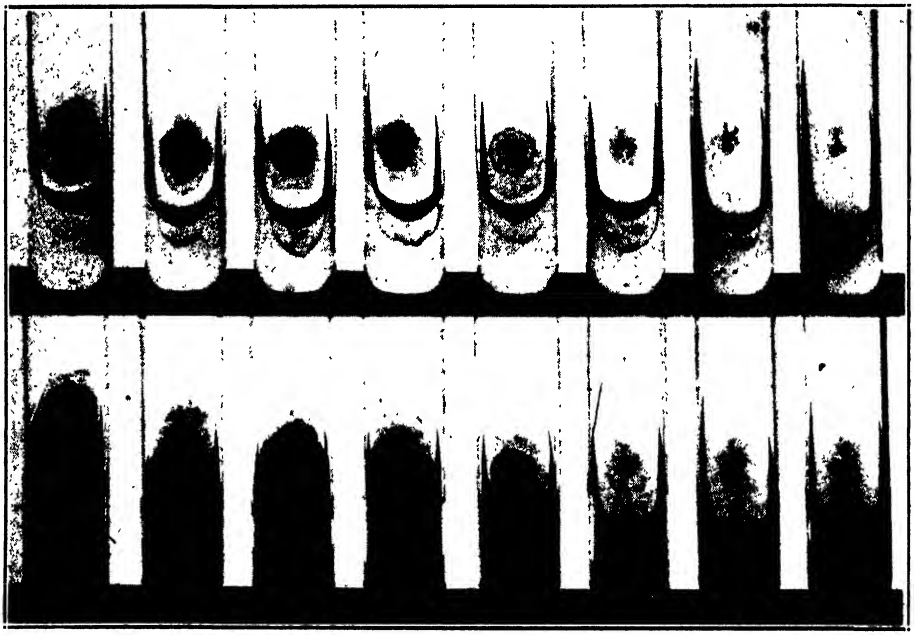


FIG. 1. Amount of pyridoxine and growth of *C. ulmi* on a mineral-dextrose medium containing asparagine and 1.5 per cent purified agar. From left to right: 25 mμ moles pyridoxine, 10 mμ moles, 5, 1.0, 0.1, 0.01, 0.001 and 0.0. Above, age 8 days; below, age 17 days.

than the liquid cultures, a comparison of pyridoxine and its analogs was made in the agar medium.

Effect of Analogs of Pyridoxine. Agar slants were prepared in triplicate with 5, 2.5, 1.0, 0.5, 0.1, and 0.0 mμ moles of pyridoxine per tube. Analogs Nos. 1421, 1422, and 3882 were used in the same amounts. For the other analogs 50, 10, 1.0, 0.5, and 0.1 mμ moles were added per tube. All tubes were inoculated from a culture of *C. ulmi* grown on thiamine-peptone agar.

The cultures were examined at from 2 to 4 day intervals over a period of more than 3 weeks.

No. 1421: No difference between the effect of pyridoxine and equi-molar quantities of the triacetate was observed when 5, 2.5, 1.0, or 0.5 mμ moles were used. However, 0.1 mμ mole was somewhat less beneficial than 0.1 mμ mole of pyridoxine.

No. 1422: There was no difference between the effect of this compound and that of pyridoxine with 5, 2.5, 1.0, 0.5, and 0.1 mμ mole per tube.

No. 1442: At the end of 10 days 50 mμ moles were beneficial, but less so

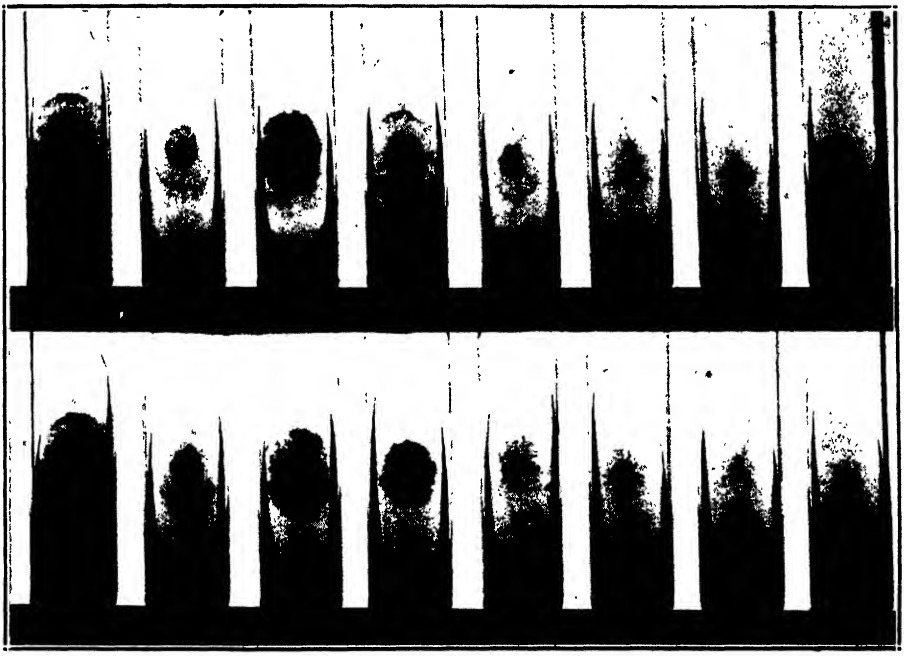


FIG. 2. Effect of analogs No. 1030 and 1032 on growth of *C. ulmi* on a mineral-dextrose medium containing asparagine and 1.5 per cent purified agar. From left to right: 0.5 mμ mole pyridoxine, 0.1 mμ mole pyridoxine, 50 mμ moles analog, 10 mμ moles analog, 1.0, 0.5, 0.1 and no addition. Above, No. 1030; below, No. 1032.

than 0.1 mμ mole of pyridoxine (fig. 3). After 23 days 50 mμ moles were less beneficial than 0.1 mμ mole of pyridoxine, 10 mμ moles had a slight beneficial effect, 1.0, 0.5, and 0.1 mμ mole had no effect. The activity of compound No. 1442 for *C. ulmi* was considered, therefore, to be less than 0.2 per cent that of pyridoxine.

No. 1443: At the end of 6 days 50 mμ moles had completely inhibited growth, 10 mμ moles gave some inhibition, 1.0 mμ mole or less had no effect. After 23 days there was some inhibition with 50 mμ moles and very slight inhibition with 10; 1 mμ mole or less had no effect.

No. 510 and *No. 511:* These had no effect, either beneficial or detrimental.

No. 1029: At the end of 6 days 10 and 50 mμ moles had completely inhibited growth but 1μ mole or less had no effect. After 23 days there was still nearly complete inhibition with 50 mμ moles, and some injury with 10. This compound was more injurious (perhaps 5 times) than No. 1443.

No. 1030: At the end of 10 days beneficial effects were noted with 0.5 mμ mole or more of this compound (fig. 2). After 23 days the growth with 50 mμ moles was about equal to that with 0.5 mμ mole of pyridoxine; 10 mμ moles of the analog were better than 0.1 mμ mole of pyridoxine; 0.5 mμ mole was better than the check, and 0.1 mμ mole had no effect. Compound No. 1030 was between 1 and 5 per cent as effective as pyridoxine.

No. 1031: At the end of 6 days complete inhibition of growth was observed with 50 mμ moles, some injury with 10, and no effect with 1 mμ mole or less. After 23 days 50 mμ moles had produced some inhibition, 10 mμ moles or less were ineffective.

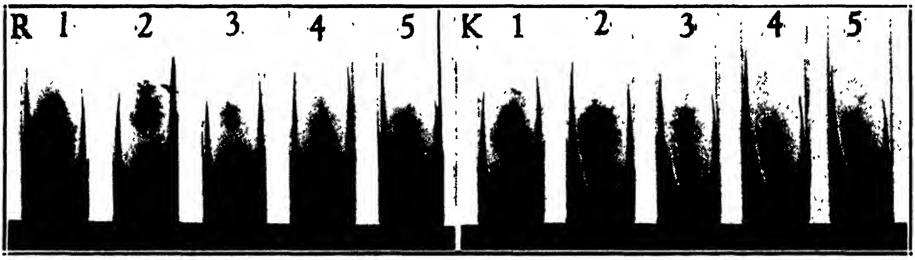


FIG. 3. Effect of analogs Nos. 1443 and 1797 on growth of *C. ulmi* on a mineral-dextrose medium containing asparagine and 1.5 per cent purified agar. 1, 0.1 mμ mole pyridoxine; 2, 50 mμ moles analog; 3, 10 mμ analog; 4, 1 mμ mole analog, 5, no addition. R, analog No. 1442; K, analog No. 1797.

No. 1032: After 10 days some benefit was noted with 0.5 mμ mole, but no effect with 0.1 mμ mole (fig. 2). At the end of 23 days 50 mμ moles were about as beneficial as 1 mμ mole of pyridoxine; growth with 10 mμ moles was nearly equal to that with 0.5 mμ mole of pyridoxine; growth with 1 mμ mole was ½ or less that with 0.1 mμ mole of pyridoxine, 0.5 mμ mole was clearly beneficial, and 0.1 mμ mole slightly. Compound No. 1032 had about 2 per cent the effectiveness of pyridoxine.

No. 1797: After 10 days 50 mμ moles were found to be beneficial, but less so than 0.1 mμ mole of pyridoxine, 10 mμ moles were better than the check and 1, 0.5, and 0.1 mμ mole were slightly poorer than the check (fig. 3). At the end of 23 days the growth with 50 mμ moles was approximately equivalent to that with 0.1 mμ mole of pyridoxine. There was little difference between the growth with 10 mμ moles and that in the check. Growth with 1.0, 0.5, and 0.1 mμ mole was somewhat poorer than in the check. Compound No. 1797 had about 0.2 per cent the effectiveness of pyridoxine.

No. 3882: This was the most detrimental of all the analogs. At the end of 6 days some inhibition was observed with 0.5 mμ mole, nearly complete inhibition with 1.0 and 10.0 mμ moles, and complete inhibition with 50 mμ moles. After 23 days marked inhibition of the spread of colony was still evident with 1, 10, and 50 mμ moles.

Antagonism between Pyridoxine and Some of its Analogs. Some growth inhibitors and some vitamins have been found to be mutually antagonistic; i.e., the injury produced by the inhibitor is reduced by addition of the vitamin or the benefit from the vitamin is decreased in the presence of the inhibitor. This relation has been found to be important in chemotherapy; some of the substances used as therapeutic agents are effective in reducing growth because of their inhibition of the action of one or more vitamins (3). The growth inhibitor may reduce the physiological availability of the vitamin by combining with it; the protein, avidin, in uncooked egg white combines with biotin and renders it inactive (2). Some inhibitors compete with the vitamin for the specific enzyme protein (sulfanilamide and para-aminobenzoic acid) (12).

Four analogs of pyridoxine tested in this investigation were found to inhibit the growth of *C. ulmi* to a greater or less extent as shown below.

Compound	Inhibition after 23 days with				
	50 mμ moles	10 mμ moles	1 mμ mole	0.5 mμ mole	0.1 mμ mole
No. 1031	Partial	None	None	None	None
No. 1443	Partial	Slight	None	None	None
No. 1029	Nearly complete	Partial	None	None	None
No. 3882	Nearly complete	Partial	Partial	None	None

To determine the effect of pyridoxine on the inhibitory action of the four analogs, 50 mμ moles of each analog were used with 50, 10, 1.0, or 0.0 mμ moles of pyridoxine in the basal medium plus 1.5 per cent purified agar. In addition 1.0 and 10 mμ moles of compound No. 3882 were used with 50, 10, 1.0, or 0.0 mμ moles of pyridoxine.

The inhibitory action of 50 mμ moles of compounds Nos. 1443, 1029, and 1031 was overcome with 1 mμ mole of pyridoxine or, to state it the other way, the beneficial action of 1 mμ mole of pyridoxine was not noticeably affected by the presence in the medium of 50 mμ moles of compounds Nos. 1443, 1029, or 1031. On the other hand, the inhibition by 50 mμ moles of compound No. 3882 was still evident in the presence of 1 mμ mole of pyridoxine, but was overcome by 10 mμ moles of pyridoxine (fig. 4).

Activity and Chemical Structure. The diacetate (compound No. 1422) was as beneficial as equi-molar amounts of pyridoxine. The triacetate (com-

pound No. 1421) was nearly equivalent to pyridoxine. A slight difference in favor of pyridoxine was noted when the effect of 0.1 mμ mole of the tri-acetate was compared with that of 0.1 mμ mole of pyridoxine; the difference, however, was slight and should be confirmed before it is considered to be significant. It seems probable that the acetates hydrolyze in solution yielding pyridoxine which accounts for their activity.

Of the ten additional analogs tested, none showed more than 5 per cent of the activity of pyridoxine; this indicates a high degree of specificity of pyridoxine for *C. ulmi*. As may be noted by comparing the structure of pyridoxine and its various analogs as given below a change of a single radical in position 2, 3, or 4 (compounds 1030, 1442, 1797, and 3882) was

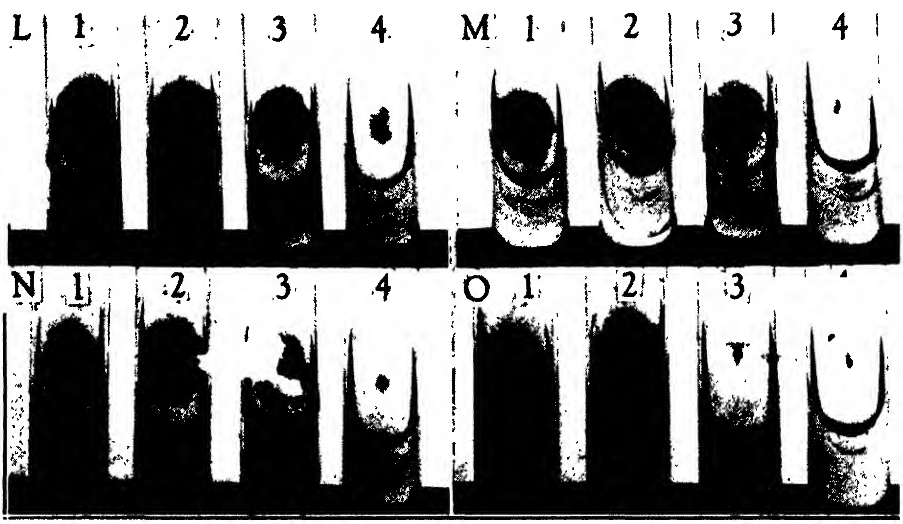


FIG. 4. Mutual antagonism of analog No. 3882 and pyridoxine in mineral-dextrose medium containing asparagine and 1.5 per cent purified agar. 1, plus 50 mμ moles pyridoxine; 2, plus 10 mμ moles; 3, plus 1 mμ mole, 4, no pyridoxine. L, no analog added; M, 1 mμ mole analog No. 3882; N, all tubes contain 10 mμ moles of analog No. 3882; O, all tubes contain 50 mμ moles analog No. 3882.

sufficient to reduce the beneficial activity of pyridoxine 95 per cent or more, or even turn it from a beneficial compound into one which inhibited growth.

It is probably not profitable on the basis of the data available here to speculate too widely on the relative importance of the various radicals of pyridoxine and how they function. The inactivity of the two lactones, compounds 510 and 511, which were neither beneficial nor detrimental, suggests that the methhydroxy groups in the fourth and fifth positions may be concerned with attachment of pyridoxine to the enzyme protein. As for those analogs which showed some benefit, the possibility of the presence of contaminating traces of pyridoxine should be borne in mind. We have no

reason to doubt the purity of the various analogs we used. Nevertheless, it must be remembered that one part of pyridoxine as an impurity in 1000 parts of the preparation of analog No. 1442 we used would have given the results obtained. There is also the possibility that contaminants account for the inhibition observed with compounds Nos. 1031, 1443, 1029, and 3882, though this is not likely because of the antagonism observed between pyridoxine and these compounds.

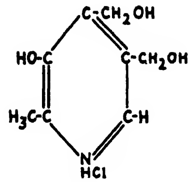
Differences in Response of Various Organisms to the Analogs. When the results obtained on the effect of the analogs on *C. ulmi* are compared with those obtained with other organisms, it seems clear that differences in the response of various organisms exist.

C. ulmi responded to some of the analogs, much as did the rats reported by Unna (4, 11). Unna found compound No. 1030 to have 10 per cent the activity of pyridoxine, No. 1442 about 2 per cent, No. 3882 about 2 per cent, and No. 1032 less than 2 per cent. We found No. 1030 to be the most favorable of the analogs for *C. ulmi*, closely followed by No. 1032; No. 1442 was the least favorable. On the other hand, No. 3882, which was slightly active for rats, inhibited the growth of *C. ulmi*. No. 1797, which was inactive for rats, had some activity for *C. ulmi*. Möller, Zinna, Jung, and Moll (5) reported compound No. 1797 to have about 2 per cent the activity of pyridoxine for *Streptobacterium plantarum* (*B. acetylcholini*). The diacetate and triacetate (No. 1422 and No. 1421) were as active as pyridoxine for *C. ulmi*, rats (11), and excised tomato roots (9), but Bohonos, Hutchings, and Peterson (1) found the triacetate inactive for *Lactobacillus lactis*.

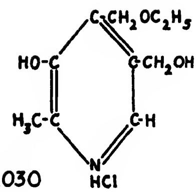
The differences in response of excised tomato roots and *C. ulmi* to some of the analogs is of interest. For example, compound No. 1030, which was from 1 to 5 per cent as active as pyridoxine for *C. ulmi*, was quite injurious to tomato roots (9). Compound No. 3882, which inhibited the growth of *C. ulmi*, was as beneficial as pyridoxine for excised tomato roots.[2] The beneficial effect of No. 3882 on excised tomato roots was confirmed by a further experiment not reported in the earlier paper. In this experiment the same sample of No. 3882 was used with tomato roots and with *C. ulmi*. It proved beneficial to the former and detrimental to the latter.

This difference in the response of tomato roots and *C. ulmi* to compound No. 3882 raises the question as to whether the same difference might not exist between *Ulmus americana* and *C. ulmi*. If it does, might not compound No. 3882 be worth investigating as a possible therapeutic agent for the Dutch elm disease? In this connection it would be desirable also to test compounds

[2] Compound 3882 was even more effective than pyridoxine on excised tomato roots when 1 or 2 mμ moles were used per culture. The growth with 10 or 50 mμ moles of the analog per culture was slower than with equi-molar quantities of pyriodoxine, but at the end of two months had reached the same level. No evidence of toxicity was observed.

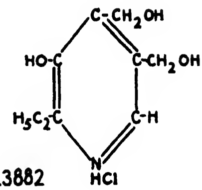


Pyridoxine



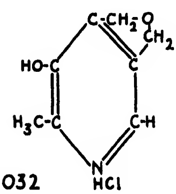
No.1030

Beneficial
1–5%
activity of
pyridoxine



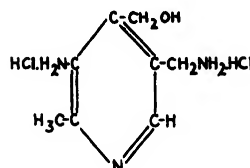
No.3882

Inhibitory
50xNo.1031



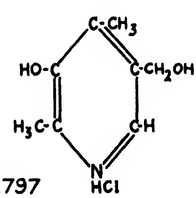
No.1032

Beneficial
about 2 %
activity of
pyridoxine



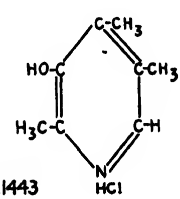
No.1029

Inhibitory
10 x No.1031



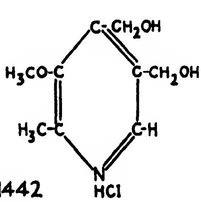
No.1797

Beneficial
about 0.2%
activity of
pyridoxine



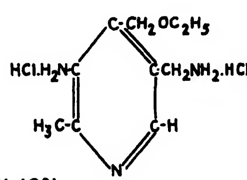
No.1443

Inhibitory
2 or 3 x No.1031



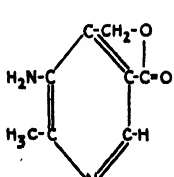
No.1442

Beneficial
below 0.2%
activity of
pyridoxine



No.1031

Slightly
inhibitory



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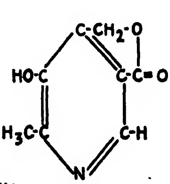

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FIG. 5. Structural formulae for pyridoxine and some of its analogs together with identification numbers and activity on *C. ulmi*.

in which the methyl radical of pyridoxine in the second position on the pyridine ring had been replaced with a propyl, butyl, or other radical. Perhaps a compound more injurious to *C. ulmi* than the ethyl pyridoxine and relatively innocuous to higher plants could be found.

Effect of Factors Z_1 and Z_2 on C. ulmi. In an earlier paper (8) we pointed out that although *C. ulmi* suffered from a complete pyridoxine deficiency, it appeared in addition to have partial deficiencies for one or more unidentified factors. This followed because greater growth occurred with the addition of malt extract to the medium than was obtained by the addition of eleven vitamins, including pyridoxine, and twenty-one amino acids. We have tested the activity of hypoxanthine and a D_R fraction[3] from potato tubers on the growth of *C. ulmi* in the presence of pyridoxine. Hypoxanthine (factor Z_1) (10) was not beneficial. The D_R fraction (containing factor Z_2) was decidedly beneficial. Between three and four times as much growth was obtained on the basal agar medium plus pyridoxine and the D_R fraction as on the agar medium plus pyridoxine alone. It appeared, therefore, that *C. ulmi* in the presence of pyridoxine suffered from a partial deficiency of factor Z_2.

SUMMARY

The effect of twelve analogs of pyridoxine on the growth of *Ceratostomella ulmi* was determined in agar culture. The diacetate and triacetate of vitamin B_6 were as beneficial as pyridoxine. Four of the ten additional analogs had 5 per cent or less of the beneficial activity of pyridoxine, two were inactive, and four inhibited growth to a greater or less extent. Pyridoxine was found to antagonize the detrimental analogs. The possibility of investigating analogs of pyridoxine as an aid in controlling the Dutch elm disease is briefly discussed.

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NEW YORK

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BULLETIN OF THE TORREY BOTANICAL CLUB
69(5): 353–359 MAY, 1942

INTERMITTENT GROWTH OF FRUITS OF CYPRIPEDIUM AND PAPHIOPEDILUM. A CORRELATION OF THE GROWTH OF ORCHID FRUITS WITH THEIR INTERNAL DEVELOPMENT

ROBERT E. DUNCAN AND JOHN T. CURTIS

Study of the growth of fruits of *Phalaenopsis* lead the authors (1942) to conclude that the three phases of growth in diameter which they found are respectively associated with proliferation of the placentae and the initiation of rudiments, maturation of the macrogametophytes, and growth of the embryos. Each two phases of growth in diameter are separated by a period of slower growth, at the first of which macrosporogenesis takes place and at the second, fertilization. The growth in length of the ovary of *Phalaenopsis* is marked by a major phase which concludes at the time of fertilization and possibly by a second at the time of embryo growth. Since ovules containing macrospore mother cells and a considerable cavity are present at the time of pollination in the ovaries of the four genera of the lady slipper orchids (*Cypripedium* L., *Paphiopedilum* Pfitz., *Phragmipedium* Rolfe, and *Selenipedium* Reichb. f.) the authors suggested that three phases of growth in diameter would be difficult, perhaps impossible, to find.

Hildebrand (1863) found that the ovules of *Cypripedium calceolus* at the time of pollination are rudimentary since their funiculi are only somewhat bent and they show just the beginnings of the inner integuments which, in unpollinated flowers, grow but slightly until the wilting of the perianth. Four days after pollination the ovules are all inverted; twelve days later the pollen tubes have reached the placentae and the inner integuments have grown over the nucelli. Fifteen days later (thirty-one days after pollination) the embryo sacs are maturing and within the next four or five days fertilization takes place. Hildebrand observed that the ovary increases in diameter by about one-fourth. His observations on *Cypripedium parviflorum* are not so complete; at least the earlier stages are similar in periodicity to those in *C. calceolus*.

Carlson (1940) in a study of seed development in *Cypripedium parviflorum* presented a schedule of events that greatly resembles that given by Hildebrand for *C. calceolus* with the exception that the various changes are, perhaps, slightly speedier. She found fertilization takes place from twenty-six to thirty-three days after pollination.

Guignard (1886) worked with tropical forms: *Cypripedium barbatum* (*Paphiopedilum barbatum*), *C. veitchianum* (*P. superbiens*), and *C. punctatum,* whose identity and habitat the authors do not know. Guignard found

these forms to be slower in development than *C. calceolus*, the temperate species with which Hildebrand (1863) worked. In general the period of time to elapse between pollination and fertilization is three or four months. Pace (1907) commented that the macrospore mother cells persisted for a long time in the ovules of the cultivated lady slipper orchids (*Paphiopedilum*).

METHODS

On May twenty-fourth, 1941, ten flowers of *Cypripedium pubescens* Willd., the large yellow lady slipper, were pollinated. The growth in length and diameter of the ovary of each flower was measured weekly with calipers to one-tenth millimeter at points, marked with India ink, designating respectively the greatest diameter at the time of pollination and the bottom and top of the ovary as shown by the termination of the superficial grooves.

The ovary of one flower of the series was fixed in Randolph's modification of Nawashin's fluid at the end of each of the first seven weeks. These fixed ovaries were imbedded and sectioned to study the internal changes. The remaining ovaries were measured at weekly intervals for six more weeks.

Likewise on May twenty-fourth two flowers of × *Cypripedium andrewsii* Fuller, Andrew's lady slipper, were pollinated. The growth of the ovaries was not measured but the stages of internal development were studied throughout the first two weeks.

On June second, 1941, several flowers of *Cypripedium reginae* Walt., the showy lady slipper, were pollinated. The growth of the ovary was followed in much the same fashion as for *C. pubescens* with the exception that no study was made of the internal development. These fruits were measured for nine weeks.

In addition to the two species and one hybrid belonging to the genus *Cypripedium*, all of which are forms native to the Madison area and were growing outdoors, three species and one hybrid belonging to *Paphiopedilum*, an Indo-Malayan genus containing the lady slipper orchids of cultivation, were studied. They were *P. villosum* (Lindl.) Pfitz. in the Botany greenhouses of the University of Pennsylvania at Philadelphia, *P. fairieanum* (Lindl.) Pfitz., *P. bellatulum* (Reichb. f.) Pfitz., and × *P. maudiae* (a hybrid between *P. callosum* var. *sanderae* and *P. lawrenceanum* var. *hyeanum*, first flowered in 1900 by Charlesworth and made repeatedly since that time) all in the greenhouses of Dr. C. K. Schubert at Madison, Wisconsin. Ovaries of *P. villosum* were removed for study of internal development seven, fourteen, twenty-one and twenty-eight weeks after pollination; ovaries of × *P. maudiae* and *P. fairieanum*, at irregular intervals. In each of these four studies the circumference rather than the diameter was measured.

RESULTS

The graph presenting the growth of a fruit of *Cypripedium pubescens*

(fig. 1) shows two periods of accelerated growth in diameter and one in length. Figure 2, a graph based on weekly increments of growth in diameter, shows two distinctly separated phases. Growth in length was finished by the

FIG. 1

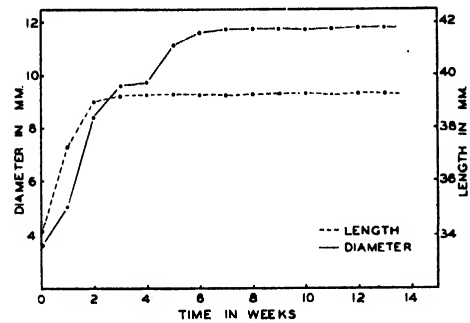

FIG. 2

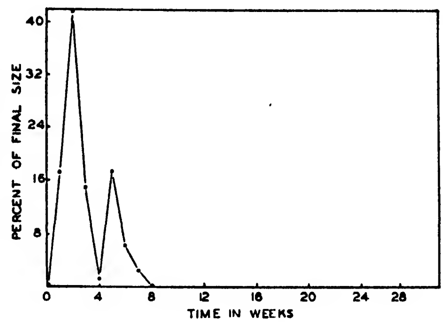

FIG 3

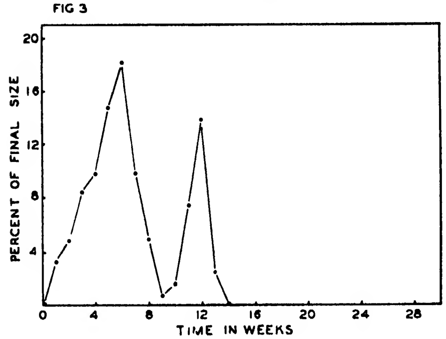

FIG 4

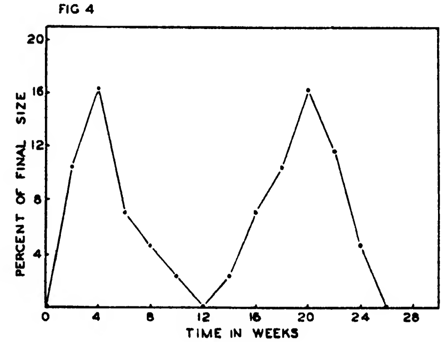

FIG 5

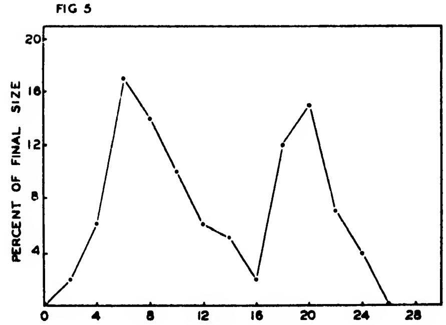

FIG 6

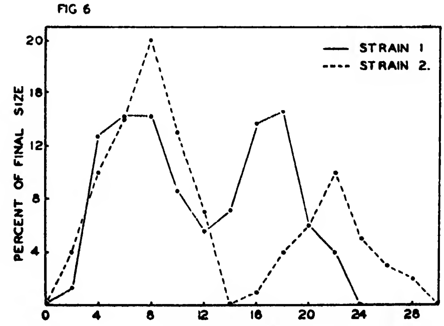

FIG. 1. Growth curve of individual fruit of *Cypripedium pubescens.* FIGS. 2–6. Increment curves of growth in diameter of *Cypripedilinae* fruits. FIG. 2. *Cypripedium pubescens* (41.1 per cent seeds possessing embryos). FIG. 3. *Paphiopedilum bellatulum* (58.8 per cent seeds possessing embryos). FIG. 4. *P. fairieanum* (63.0 per cent seeds possessing embryos). FIG. 5. *P. villosum* (32.3 per cent seeds possessing embryos). FIG. 6. × *P. maudiae* (percentage of seeds possessing embryos not determined).

end of the fourth week. The growth of fruits of *C. reginae* showed a similar periodicity.

An ovule of *C. pubescens* at the time of pollination possesses a distinct macrospore mother cell and the rudiments of the inner integument; the funiculus is generally somewhat bent. By the end of the first week the funiculus has completed a curvature of about ninety degrees, the inner integument has grown approximately one-half way over the nucellus, and occasionally the rudiments of an outer integument can be detected. The nucleus of the macrospore mother cell has enlarged and is in an early prophase of the first of the reduction divisions. There is some variation from ovule to ovule; the most frequently observed stage is leptonema.

At the close of the second week pollen tubes are growing in the ovary cavity, the majority lying behind the placentae but the tips of a few growing over the branches of the placentae. The ovules have completed their inversion. The nucellus is completely covered by the inner integument, which in turn is sheathed for one-half its length by the outer integument. The majority of the macrospore mother cells are in various later stages of the first of the reduction divisions. In a few cases metaphase of the second of these divisions and even binucleate macrogametophytes have been observed. Over this period of time, which is as far as studies on × *C. andrewsii* were carried, the development of the ovules in × *C. andrewsii* and *C. pubescens* are exactly parallel.

During the third week the outer integument of the majority of the ovules grows out until it has nearly reached the apex of the nucellus. Most of the pollen tubes are growing over the placental extremities. Almost all the ovules contain macrogametophytes of varying degrees of complexity from binucleate to practically mature. At the close of the fourth week most of the macrogametophytes are mature; in some fertilization has taken place. At the close of the following week few-celled embryos are present in some ovules, although zygotes are more common. When the ovule contains a young embryo, it is decidedly elongated. Throughout the next two weeks in which the internal changes were followed the embryos grew in size and the masses of pollen tubes disappeared from the ovary cavity. No growth in diameter was detected after the eighth week. The ripening of the seed was not followed.

The fruit wall does not change enough in thickness, while the ovary is maturing, to account for any appreciable amount of the increase in diameter of the fruit. The increase is mainly in the amount of ovary cavity which is provided by the periclinal elongation and enlargement of the cells of the ovary wall. No division figures were found in any of these cells.

The graphs portraying the increments of growth in diameter of the ovaries of *Paphiopedilum bellatulum* (fig. 3), *P. fairieanum* (fig. 4), *P. villosum* (fig. 5), and × *P. maudiae* (fig. 6) all posses two phases.

Seven weeks after pollination the ovules of *Paphiopedilum villosum* vary from those containing macrospore mother cells in some phase of meiosis through those having early macrogametophyte stages. Fourteen weeks after

pollination zygotes are present in the ovules; after twenty-one weeks the seeds are distended by the embryos within but are not mature. The seeds from the twenty-eight-weeks-old fruit are capable of germination although the fruit is not ripe.

Both *Paphiopedilum fairieanum* and × *P. maudiae* possess ovules with slightly bent funiculi and with the beginnings of inner integuments at the time of pollination. One hundred days after pollination the ovules from a fruit on one plant of × *P. maudiae* are inverted, the nucellus of each being cloaked with the inner integument and the beginning of the outer, and the macrospore mother cells being in some stage of reduction division. Thirty-seven days later a fruit from another plant contains immature seeds. Figure 6 illustrates the fact that the development of fruit on various plants of × *P. maudiae* of different origin vary considerably, particularly in concluding the first phase of growth and the amount of growth taking place throughout the second phase. Crosses within × *P. maudiae* generally result in a low percentage of viable seeds.

DISCUSSION

While the ovary of *Cypripedium pubescens* is in the first of its two phases of growth in diameter and its only detected phase of growth in length, the ovules are passing from the stage in which they possess macrospore mother cells to one in which they possess mature macrogametophytes. Following this growth phase there is a well-nigh complete cessation of growth in both length and diameter. During this period fertilization is taking place at its maximum rate; at its close ovules containing zygotes or few-celled embryos are found. While the embryos are growing rapidly and the ovules have elongated immediately after fertilization, the second phase of growth in diameter occurs. These statements are likewise true for the forms of *Paphiopedilum* studied. In *Phalaenopsis* a second phase of growth in length, of small extent, is sometimes detected.

Phalaenopsis has three phases of growth in diameter, these phases having to do with placental proliferation and ovule rudiment initiation, preparation of the ovule for fertilization, and seed maturation. Macrosporogenesis occurs during the period of decelerated growth between the former two, and fertilization at a similar time between the latter two. The effect of the growth regulatory substances present in the pollen is added to the first growth phase. In *Cypripedium* macrospore mother cells are present at the time of pollination, a process which immediately initiates a phase of growth in both length and diameter. Hence macrosporogenesis, which in *Phalaenopsis* occurred during a period of relatively little growth, takes place during the first growth phase in *Cypripedium* and *Paphiopedilum*. This phase culminates when the ovules are ready for fertilization which occurs in *Cypripedium*, just as in *Phalaenopsis*, during a period of decelerated growth of the ovary. Thus

Cypripedium meets the authors' expectation that only two phases of growth in diameter would be present. The observations of Tukey (1933) on cherry point out, as far as internal development is concerned, the presence of two phases of ovary changes prior to the opening of the flower. These are a gradual growth of both ovary and ovules up to fourteen days before full bloom and a grand phase of ovule growth up to the time of full bloom. Pollination takes place while the ovules are developing rapidly. Macrospore mother cells are present at the close of the first phase; during the second the macrogametophytes mature. It is interesting to note that a flower of *Cypripedium* or *Paphiopedilum* opens at a stage, relative to its internal development, that compares with the comparatively quiescent state which an ovary of *Phalaenopsis* reaches after completing the first phase of its growth.

The type of flower found in *Phalaenopsis* and many other tropical orchids which are in full bloom when the placentae have reached a stage short of proliferating, the type found in *Paphiopedilum*, *Cypripedium*, and many genera of temperate orchids (Hildebrand 1863) whose perianth expands when macrospore mother cells are present in the ovules, and the more frequent Angiosperm type in which ovules are mature or almost mature at the time of flower expansion form a series in which the authors believe that three major types of development subsequent to pollination can be recognized. In the type to which *Phalaenopsis* belongs three phases of growth in diameter will be found; in the type to which the *Cypripedium* complex belongs, two; and in the more usual Angiosperm type, one. Each phase immediately following pollination is compounded with a pollination effect which may or may not be recognizable. After fertilization the breaks in the growth curves, such as are found in drupaceous fruits, are modifications of the last phase of growth. The slightly bimodal curve for the first phase of growth in diameter of *Phalaenopsis schilleriana* as determined by the authors (1942) indicates that at least the first major phase of growth in diameter likewise may be modified.

SUMMARY

The ovaries of both *Cypripedium* and *Paphiopedilum* possess two phases of growth in diameter and one in length. The first phase of growth in diameter and the phase of growth in length take place while the ovules are maturing prior to fertilization. The second phase of growth in diameter takes place while the embryos are growing most rapidly. Fertilization takes place at a time when growth of the ovary has almost ceased.

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STUDIES ON THE EMBRYO OF HORDEUM SATIVUM—II. THE GROWTH OF THE EMBRYO IN CULTURE

JAMES MERRY

INTRODUCTION

In a preceding paper (Merry 1941) the development of the embryo of *Hordeum* was described and the morphology of the embryo was interpreted, in part, from plants which had been grown from young embryos of various ages. The cultured plants themselves were not described and it is proposed to give here an analysis of the growth of the embryos in culture. Embryos of various ages were transferred to an artificial medium, and at five-day intervals, over a period of a month, one or two plants of each age group were removed from culture. These plants were studied histologically and compared with normal embryos of the same age as those from which they were grown. The plants from different ages of embryos were compared in size and in the degree of differentiation.

HISTORICAL SURVEY

Many workers have grown excised embryos of plants on artificial media, for a variety of reasons. Bonnet (1754), Stingl (1907), Dubard and Urbain (1913), Buckner and Kastle (1917), and Andronescu (1919) grew isolated embryos of mature seeds in order to show that they could germinate independently of the rest of the seed, particularly of the stored food. Bonnet was the first to study the germination of embryos from which the cotyledons had been detached; he worked with *Phaseolus multiflorus*. Buckner and Kastle (1917) grew mature embryos of lima bean with the cotyledons removed. Stingl (1907) found that the mature embryos of certain species of grains, when transferred to the endosperm of other species, grew as well as or better than when put on the endosperm of the same species. Dubard and Urbain (1913) concluded that seedlings could be grown from isolated embryos of seeds with abundant endosperm, but that, in seeds in which the food was stored in cotyledons, removal of these prevents the development of the seedling. Andronescu (1919) found that plants grown from excised corn embryos would grow to maturity, but that the plants were smaller and had fewer leaves and ears than plants from whole grains. Essenbeck and Sussenguth (1925) grew excised embryos of mature corn grains to study the enzymes in the germinating embryos. Hannig (1904), Dietrich (1924), White (1932), and La Rue (1936) grew embryos from immature fruits of various ages largely to determine its feasibility and to discover how small an embryo could be grown in culture. They found that the embryos grew

into small plants rather than continuing to develop as embryos, and that the smallest embryos used did not grow at all. This minimum size varied with the kind of plant used. Tukey (1933) and others have used this technique to obtain plants which cannot be grown from mature seeds. In using the method for this purpose some of the workers have not removed the parts of the ovule surrounding the embryo, since it was only desired that the embryo germinate and produce a plant. Tukey (1938) has also developed this technique as a method for testing the viability of seeds, and for the study of embryos of certain fruit trees. La Rue and Avery (1938) studied the development of excised embryos of *Zizania* in culture and compared their growth with that of the embryo in normal development. They described the plants after five days in culture and compared them with embryos of the same size as those from which the plants were grown. They found that the growth of the scutellum and the epiblast was arrested, and that small embryos took longer to reach the "seedling stage" than large ones. The smallest embryos did not reach the seedling stage at all.

MATERIAL AND METHODS

The material was the same as that used in the first paper of this series (Merry 1941). Heads whose age after fertilization had been determined were used. Two or three ovules were removed and fixed for the study of normal embryos. The embryos were removed from the remaining ovules and placed in Petri dishes on 0.8 per cent agar containing 2 per cent sucrose and Shive's solution R5S2 (Miller 1931) diluted to one-fifth. According to Harlan and Pope (1926), "if the florets of the four basal and four terminal nodes are discarded, the remaining kernels are of the same age." These basal and terminal florets were not used and the embryos of the remaining florets were found to be very nearly of the same age, which was determined only to within a day. The cultured embryos were usually transferred after five days and one or two of them killed and fixed at that time unless it was desired to save the culture for later stages. Usually there were enough embryos in one head to supply one or two plants every five days for a month, thus giving a developmental series of plants for that particular age of embryo. Such series of plants were obtained from embryos of many different ages.

The embryos and cultured plants were cleared and mounted temporarily in xylene and drawn for record purposes. They were then embedded, sectioned, and stained as described by Merry (1941). Large structures such as roots and leaves were measured directly with a ruler. Other measurements were made with a microscope and ocular micrometer from temporary mounts and sections.

DESCRIPTION OF RESULTS

In the description which follows, after a brief description of the growth

of the cultured embryo as a whole, the growth of the various parts is taken up in detail. The plants from excised cultured embryos are compared with normal embryos of the same age as the excised embryos. Such a comparison reveals the change which took place in the excised cultured embryo.

Growth of the Embryo as a Whole. Usually when the embryos of barley were excised and placed on the culture medium, the coleoptile elongated, the stem meristem grew out of it producing new leaves, and the root meristem grew out of the coleorhiza. There was, however, some variation in this response, depending upon the age of the embryo. The youngest embryos cultured were from seven to eight days old and showed no change after being in culture for two months. The addition of vitamin B_1 to the medium made no difference in the response of these embryos, though they were kept in both light and dark. Embryos nine days old showed considerable cell enlargement, but none of the cells continued to divide. The 10-day-old embryos underwent some cell division but ceased growing after 15 days. There was more cell division in the 11-day-old embryos, but within a month all division in these embryos had ceased. In some of the 11-day-old embryos a slight germination of the primary root meristem occurred after from fifteen to twenty days in culture, while in 12-day-old embryos it germinated after five days and grew to considerable length. In the stem meristem of the 12-day-old embryos divisions were still occurring after a month in culture. The growth of all older embryos was essentially the same as that of the 12-day-old embryos except for increase in size and growth rate.

Growth of the Scutellum. No cell divisions were observed in the scutella of cultured embryos examined five days after excision, regardless of the age of embryo used. The scutella of embryos 9 days old or older enlarged when the embryos were cultured and measurements of the cells of the scutella showed that they had enlarged proportionally; hence only a few cells could have divided. The increase in the size of the scutella of embryos in culture was slight in comparison with the increase in size of the other parts of the embryos and was not restricted to one dimension (figs. 1, 2). From figure 2 it can be seen that the ratio of the final length or width of the scutellum of cultured embryos to the length or width of the scutellum of comparable normal embryos decreased as the age of the embryos increased. This ratio is greater than 2.0 for the youngest embryos and is about 1.2 for the oldest embryos.

In embryos up to 14 days old the epidermal and subepidermal cells on the back of the scutellum did not enlarge in culture so much as those on the front, so that the scutella of such embryos in culture curved away from the coleoptiles. In the scutella of embryos 15 or more days old the cells all enlarged to the same extent, so that the scutella remained straight.

The vascular bundles of the scutellum began to differentiate in the normal 10-day-old embryo as two strands of elongated cells. In culture these cells retained their difference in shape and in some scutella became lignified after a month. After five days in culture the scutellar bundles of embryos 12 or more days old became lignified, the lignification gradually becoming heavier.

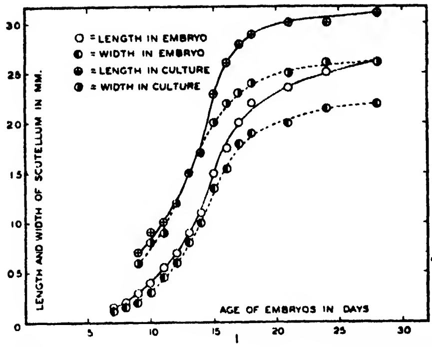
In the normal development of the embryo the epidermal cells which are to become the epithelial layer of the scutellum divide more than the other cells of the scutellum so that they become proportionally smaller. In culture the cells of the epithelial layer became more vacuolate and enlarged to the same extent as did the other cells of the scutellum.

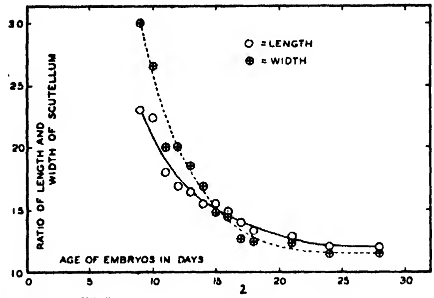
Growth of the Coleoptile. Some cell divisions were observed in the coleoptile of the 10-day-old embryos in culture, and the frequency of cell divisions increased with increase in the age of the embryos. In culture cell divisions occurred during a period of slightly more than five days, and in the older embryos this period became shorter. A period of cell enlargement naturally followed. In the 9-day-old embryos the cells enlarged in all dimensions, while in older embryos the enlargement was mostly in length. In the 11-day-old embryos the cells of the coleoptile increased from about twice their original length after five days to a final length about ten times the original after fifteen days. The final length to which the coleoptile of embryos of various ages grew increased with an increase in the age of the embryo (fig. 3). The ratio of the final length in culture to the length in the normal embryo increased rapidly from 9-day-old to 13-day-old embryos and then gradually decreased as the age of the embryos increased (fig. 4).

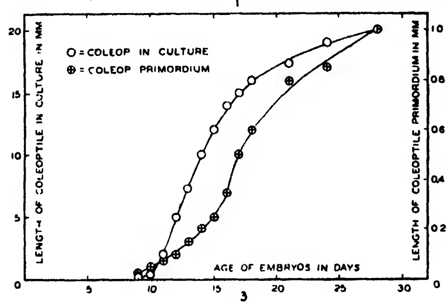
The vascular bundles of the coleoptile were first differentiated in plants from 11-day-old embryos, and after a month those in some of the plants contained lignified elements. The bundles in the coleoptiles of 12-day-old embryos were lignified after five days in culture, as were those of older embryos. As the age of the embryos increased the number of elements in the bundles increased, and in plants from embryos 15 or more days old bundle sheaths were formed around the bundles.

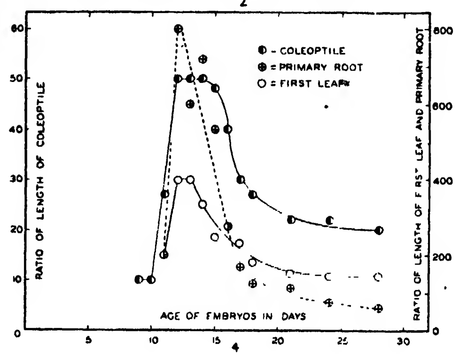
In the normal embryonic development of the coleoptile an opening is at first present at the tip and soon becomes located on the front of the coleoptile. This pore gradually becomes smaller until the sides touch and the coleoptile forms a complete sheath around the plumule. When embryos from 10 to 13 days old were cultured, the elongation of the coleoptile stretched the pore so that in some embryos it was about as long as the coleoptile itself. In the plants from older embryos the pore was not enlarged so much by the growth of the coleoptile, but it was split by the growth of the plumule.

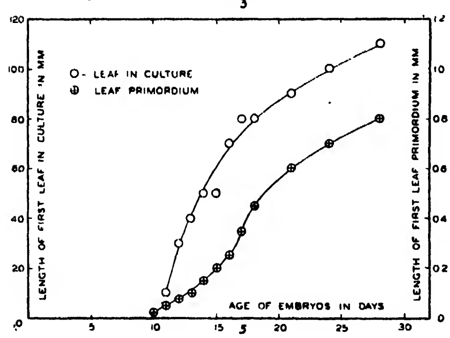
Growth of the Stem Meristem. In the stem meristem of 9-day-old embryos in culture for five days no cell divisions were observed. Divisions











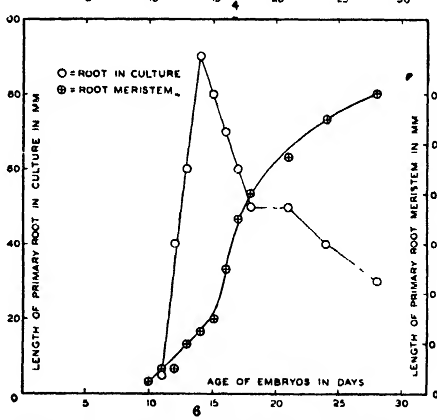


Fig. 1. Length and width of the scutella of normal embryos of different ages and the final length and width of scutella of embryos of corresponding ages excised and grown in culture for 30 days. **Fig. 2.** Ratio of the final length and width of the scutella of excised embryos grown for 30 days to the length and width of the scutella of normal embryos of the same age as the excised embryos. **Fig. 3.** Length of the coleoptile primordia of normal embryos of different ages and the final length of coleoptiles of embryos of corresponding ages excised and grown in culture for 30 days. **Fig. 4.** Ratio of the final length of the coleoptiles, primary roots, and first leaves of excised embryos grown for 30 days to the length of the primordia of these parts in normal embryos of the same ages as the excised embryos. **Fig. 5.** Length of the first leaf primordia of normal embryos of different ages and the final length of the first leaf of embryos of corresponding ages excised and grown in culture for 30 days. **Fig. 6.** Length of the primary root meristems of normal embryos of different ages and the final length of primary roots of embryos of corresponding ages excised and grown in culture for 30 days.

were observed in 10-day-old embryos after five and after ten days but not after fifteen. In the plants from 11-day-old embryos cell divisions in the stem meristem had not stopped until after a month in culture. In plants from embryos 12 days old or older the cell divisions were found in stem meristem after a month in culture.

No cell enlargement occurred in the meristem of 8-day-old embryos in culture, but in embryos 9, 10, and 11 days old the cells of the meristem enlarged considerably after division had ceased. In some embryos this caused the meristem to protrude beyond the coleoptile and the first leaf primordium. In embryos 12 or more days old the cell number and the size of the stem meristem remained about the same during the growth in culture regardless of the age of the embryo when cultured. There was of course some variation in size and cell number at different points in the plastochron.

TABLE 1

Number of leaf primordia formed in the stem meristem

Age of embryos in days	Days in culture						
	0	5	10	15	20	25	30
9	0	1	*	....	. .	......	.. ..
10	1	1	2	*	.	.. .	..
11	1	2	2	3	3	3	*
12	1	2	3	3	4	4	4
13	2	2	3	4	4	4	5
14	2	3	4	4	5	5	5
15	2	3	4	4	5	5	5
16	3	4	4	5	5	5	6
17	3	4	4	5	5	6	6
18	3	4	5	5	5	6	6
21	3	4	5	......	....	.....	......
24	3	4	5	....	.. .	......	......
28	3	4	5	.....	.....	......	.....

* Plants stopped growing.

Table 1 shows the time at which each of the first five leaf primordia were formed from various ages of embryos. It can be seen from this table that at some ages the difference of a day in the age of the embryos when cultured made several days difference in the time at which a particular leaf primordium was formed, while at other ages it made little difference.

Growth of the First Leaf. The time of formation of the first leaf primordia has been stated above. Many cell divisions were observed in the first leaf in cultured plants, most of them in the basal region. In plants from embryos up to 14 days old divisions were observed in the first leaf after 5 and after 10 days, in those from older embryos after five days but not later. Most of the elongation of the leaf took place during the first 10–15 days in

TABLE 2

Growth of the first leaf in culture

Age of embryos in days	Length of first leaf primordium in embryos in cm.	Length in cm. of the first leaf of plants grown in culture					
		5 days	10 days	15 days	20 days	25 days	30 days
11	0.005				1	1	1
12	0.008		0.5	1	3	3	3
13	0.010		1.5	3	4	4	4
14	0.015	0.5	3.0	5	5	5	5
15	0.020	0.5	3.0	5	5	5	5
16	0.025	3.0	7.0	7	7	7	7
17	0.035	4.0	8.0	8	8	8	8
18	0.045	4.0	8.0	8	8	8	8
21	0.060	4.0	8.0	9	9	9	9
24	0.070	4.0	9.0	10	10	10	10
28	0.080	5.0	10.0	11	11	11	11

culture (table 2). The period of elongation became shorter in the plants from older embryos. The length finally reached by the first leaf increased with an increase in the age of the embryos when cultured, as is shown in figure 5. The ratio between the final length of the first leaf in culture and the length of the first leaf primordium of the normal embryo increased rapidly from 11- to 12-day-old embryos and then gradually decreased (fig. 4).

The number of vascular bundles formed in the first leaf of plants grown from embryos of different ages is shown in table 3. The number of bundles

TABLE 3

Differentiation of bundles in first leaf

Age of embryos in days	Number of bundles in first leaf primordium in embryos	Number of bundles in first leaf of plants in culture			
		5 days	10 days	15 days	30 days
10	0	0	1	3	3
11	0	1	3	5	7
12	1	3	5	7	7
13	3	5	7	7	7
14	3	7	13	13	13
15	7	13	13	13	13
16	13	17	17	17	17
17	15	19	19	19	19
21	19	19	19	19	19
28	19	19	19	19	19

in the first leaf of any plant was greater than the number differentiated in the normal embryo from which it was grown, except for the oldest embryos, in which all the bundles present in the mature first leaf of a seedling are

differentiated. The number of bundles in the mature first leaf of the cultured plants varied with the age of the embryos from which they were grown (table 3).

Growth of the Roots. The time of differentiation of the seminal roots in the normal embryos and of their germination from the cultured embryos is shown in table 4. No seminal roots were formed in culture unless they had

TABLE 4

Growth of seminal roots

Age of embryos in days	Number of root meristems in embryos	Number of seminal roots on plants grown in culture				
		5 days	10 days	15 days	20 days	30 days
11	1	0	0	1	1	1
12	3	1	1	3	3	3
13	3	3	3	3	3	3
14	3	3	3	3	3	3
15	5	3	3	5	5	5
16	5	3	5	5	5	5
17	7	5	5	5	5	7
18	7	5	5	7	7	7
21	9	5	7	7	7	9
24	9	7	7	7	9	9
28	9	7	9	9	9	9

begun to differentiate before the embryo was removed from the ovule. No seminal root germinated so rapidly from an embryo in which it was just differentiated as did the seminal root in the same position from an older embryo.

The length of the primary root at 5-day intervals on plants from embryos of various ages is shown in table 5. The length stated represents an average

TABLE 5

Growth of primary root

Age of embryos in days	Length of primary root meristem in embryos in cms.	Length of primary root of plants grown in culture					
		5 days	10 days	15 days	20 days	25 days	30 days
11	0.005	......	.. .	.....	.. ..	0.5	0.5
12	0.005	0.15	0.5	2	2.0	2.5	4.0
13	0.010	0.25	1.0	2	2.5	4.0	6.0
14	0.013	1.0	2.5	5	6.0	7.0	9.0
15	0.015	2.0	3.0	5	5.0	7.0	8.0
16	0.025	2.0	3.0	5	5.0	7.0	7.0
17	0.035	2.0	3.0	5	5.0	5.0	6.0
18	0.040	2.0	3.0	5	5.0	5.0	5.0
21	0.048	2.5	3.0	3	4.0	4.0	4.0
24	0.048	2.5	4.0	4	4.0	4.0	4.0
28	0.060	2.0	3.0	3	3.0	3.0	3.0

of only a few plants, and the variation from one plant to another is great. The data in table 5 and in figure 6 show that the plants from embryos about from 14 to 16 days old had longer primary roots than those from embryos of any other age. The curve in figure 6 would be the same if the maximum length attained from an embryo of each age had been plotted; the values would of course be higher. The ratio of the final length of the primary root of the cultured plants to the length of the primary root meristem in the normal embryos increased very greatly from the 11- to 12-day-old embryos and then gradually decreased (fig. 4).

The growth of the first pair of seminal roots was similar to that of the primary root, though they never became as long as it did. The other seminal roots showed a continuous increase in final length with an increase in the age of the embryos. In the oldest embryos the first three pairs of seminal roots grew to about the same length as the primary root of the same plant. After 15 days in culture adventitious roots were formed on the plants from embryos from 12 to 16 days old, and on plants from embryos 24 days old or older after about 20 days in culture.

Growth of the Coleorhiza and Suspensor. The cells of the coleorhiza enlarged to about twice their original diameter in all embryos nine days old or older. This enlargement took place in less than five days, after which the cells tended to collapse. In the older embryos the cells elongated more in the long axis of the embryo than in other dimensions. Only a few scattered divisions were observed in the coleorhizae of normal embryos, and none was seen in those of cultured plants after five days in culture.

In most of the embryos which grew in culture the suspensor did not change at all. In some of the very youngest embryos the cells of the suspensor enlarged to the same extent as the other cells.

DISCUSSION

Compared with the growth of the embryo in normal development the growth in culture is strikingly different and more closely resembles that of a seedling. This has been noted by all workers who have grown immature embryos on artificial media. One basic difference in the two types of growth is that in culture the cells of the various parts of the embryo undergo considerable enlargement, while in normal development the cells tend to remain the same size or even to become smaller. Whatever change in the conditions causes the change in the manner of growth may be one which removes the check on cell enlargement which appears to exist in normal development. However, not all the cells in all the embryos cultured tend to enlarge. In the youngest embryos cultured no cell enlargement is observed. In slightly older embryos all the cells enlarge, and in still older embryos the cells do not enlarge in certain regions which remain meristematic.

This suggests a second general difference between the two types of growth: in culture cell division becomes more localized than in normal development. Certain regions are differentiated in the normally developing embryo in which the cells are smaller and divide more frequently than those in the rest of the embryo. But the other regions in the normal embryo show scattered cell divisions during development which cease when the embryo is removed from the ovule and is placed in culture. The continued growth of the embryo in culture is restricted to those regions in which cell division continues. It appears then that the change or changes in conditions due to excision and culturing, which cause the change in manner of growth, inihibit cell divisions in most of the embryo and lead to cell enlargement in some parts.

As has been indicated above there are also differences in growth in culture between embryos of different ages. Such differences have been observed before. Hannig (1904) and all workers in this subject since this time have found that there is a minimum size at which the embryo can be removed and grown in culture. This is not the same size for all plants nor in all investigations. In culturing immature embryos of certain fruit trees Tukey (1938) observed that each stage of embryo which was cultured gave a different "growth pattern." The variation was largely in the presence or absence of roots and in the degree of elongation of the stem. If we make allowance for the difference in the material used, the various "growth patterns" shown by Tukey (1938) are surprisingly similar to the variation between the plants grown from different ages of barley embryos. The cells in the very smallest barley embryos neither enlarged nor divided. This may possibly be because the cells are unable to carry on normal metabolism in the presence of oxygen in so high a concentration as that in air. It seems reasonable to assume that in the ovule the oxygen concentration is not as high as in air. In slightly older embryos the cells are able to enlarge, but do not continue to divide, so that the growth of the plant is terminated. It is felt that the enlargement of the cells in culture may be related to exposure to an oxygen concentration higher than that in the ovule, since in embryos in which the opening in the coleoptile exposes the stem meristem to the air, the cells in the meristem enlarge. In slightly later stages, when the pore is closed, the cells in the meristem remain small and continue to divide. This may of course be due to changes in the cells of the meristem themselves, to the effect of pressure on the meristem, or to the increase in the size of the embryo and in its ability to supply food to the meristem.

The final size of the cultured plants increases with the increase in the age at excision of the embryos from which they are grown. This is correlated with the increase in the size of the embryos from which the cultured plants were grown and to their greater differentiation, but if the final size of the

various parts of the cultured plants is compared with the size of these parts in normal embryos of the same age as those from which the plants were grown, it is seen that the difference is not all explained directly by the difference in the size of the normal embryos. The ratio of the final size of the parts in cultured plants to that in normal embryos increases very rapidly with the age of the embryo for very young ages but soon decreases with the increased age of the embryos.

The variation in the size of the primary root with increase in the age of the embryos is most interesting. The final root length increases rapidly with an increase in the age of the embryos from 11 to 14 days, and then decreases in plants from progressively older embryos. This is doubtless due to competition with the other seminal roots. Apparently some factor which is necessary for the growth of the roots is present in a limited amount, so that the more roots formed the smaller each is. At the same time the total length of roots formed appears to increase with the age of the embryos, which may mean that there is an increase in the controlling factor as a young embryo becomes older.

The difference in the number of seminal roots produced on plants from different ages of embryos is the most obvious morphological difference in the material. This is clearly related to the degree of differentiation in the normal embryo at the time of excision. Only as many seminal roots as are already formed in the normal embryo will grow in culture. The other roots produced in culture are adventitious roots formed on the stem.

The number of vascular bundles in the leaves of cultured plants is another morphological character which varies with the age of the embryo when excised. Embryos about 21 days old have the maximum number of bundles differentiated in the first leaf primordium, and the first leaf of the plants cultured from them has the same number. All plants from embryos younger than 21 days have more bundles in the first leaf than there were in the first leaf primordia of the embryos from which they were grown. Evidently some differentiation takes place in the first leaf in culture, though not so much as usually takes place in the normal development of the embryo. Plants from very young embryos have fewer bundles in the first leaf than plants from mature embryos. It would be interesting to know if the leaves differentiated after the embryo was excised would have the same differences in size and number of bundles for different ages of embryos as the first leaf has. Since most of the plants were not allowed to grow long enough for more than the first two leaves to mature, this point could not be determined.

The differentiation of the leaf primordia also varies with the ages of the embryos when excised. They are formed more rapidly from the older embryos. Also the leaves elongate and their bundles lignify sooner and to a greater extent in the older embryos. This is probably related in a complex

manner to the increased size and greater differentiation of the older embryos. The increase in size may mean that there is more food in the embryo, so that growth could take place more rapidly. Also, since the root meristems are more numerous and the leaf primordia larger, the plants from the older embryos would produce roots and leaves, and therefore their own food supply, sooner and in larger amounts. Factors other than food supply are probably involved and will be better understood after a physiological study of the normal development of the embryo and its growth in culture has been made.

This investigation was begun while the author was an assistant in the Department of Botany, University of Michigan, and was completed while he held the Emma Cole Fellowship in Botany from the same institution. The writer wishes to express his appreciation of the support which has made this work possible. It is a pleasure to acknowledge the suggestions and criticisms of Professor C. D. La Rue of the University of Michigan, under whose guidance this work was done, and the generous assistance of Professor David R. Goddard, of the University of Rochester, in the preparation of the manuscript.

SUMMARY

1. When embryos seven or eight days old were cultured on a nutrient agar medium no change was observed.

2. When embryos nine days old were cultured all the cells enlarged and no cell division occurred.

3. When embryos ten or eleven days old were cultured some cell division occurred, but eventually all the cells stopped dividing and enlarged.

4. When embryos twelve days old or older were cultured the cells in certain regions continued to divide as long as the plants were grown. The cells in other regions enlarged at once or divided for a short time and then enlarged.

5. No cell division and only slight cell enlargement was observed in the scutellum of cultured embryos.

6. The size of the stem meristem was about the same in all plants from embryos twelve or more days old.

7. The leaf primordia were formed more rapidly in plants from older embryos.

8. The coleoptile and leaves were larger in plants from older embryos.

9. The ratio, of the size of the coleoptile, leaves, and roots of cultured plants to the size of these parts in normal embryos, increased rapidly with the age of the embryos for early ages but soon decreased with an increase in the age of the embryos.

10. The vascular bundles became lignified sooner and to a greater extent in plants from older embryos.

11. The primary root was longest in plants from embryos from 14 to 16 days old and was successively shorter in plants from progressively older embryos.

12. Only as many seminal roots as were already differentiated in the embryo grew in culture. Other roots formed were adventitious roots on the stem.

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NORTH AMERICAN RANUNCULI—V

LYMAN BENSON

The first four articles in this series deal with the sections of the subgenus *Euranunculus*, as follows: I and II, sect. *Chrysanthe;* III, sects. *Echinella* and *Epirotes;* IV, sects. *Flammula* and *Hecatonia.* This concluding article is a treatment of the other subgenera. The area included in the series is North America north of Mexico. The delimitation of sections and subgenera was published in a paper entitled "The North American Subdivisions of *Ranunculus*" (Am. Jour. Bot. **27**: 799–807. 1940). Detailed specific descriptions are reserved for the North American Flora.

In order to facilitate use of this series of articles and its reprints in identification of specimens, the keys to the subgenera and sections published previously in the American Journal of Botany (*loc. cit.*) are reproduced at the beginning of this concluding article.

KEY TO THE SUBGENERA

Sepals 5 or rarely 6, except in *R. alveolatus* (*Euranunculus* sect. *Flammula*) which has simple, entire leaves and petals only 2–2.5 mm. long; achenes not constricted; cotyledons 2.

- Achenes or utricles not transversely ridged, except in *R. sceleratus* (*Euranunculus* sect. *Hecatonia*) which has 40 or commonly 100–300 minute beakless achenes in an elongated head; petals usually glossy, yellow or rarely red or white.
  - Sepals deciduous during or soon after anthesis; fruits not utricular, 1-chambered.
    - Pericarp not striate or nerved, thick and firm. . Subgenus I. *Euranunculus.*
    - Pericarp striate, the nerves 3 or more on each face, these sometimes branched, the ovary wall thin and usually fragile at fruiting time. . ... Subgenus II. *Cyrtorhyncha.*
  - Sepals persistent in fruit; fruits utricular or 3-chambered.
    - Annual; hypocotyl simulating a tap-root; fruit with one basal seed-chamber and two lateral empty vesicles, the beak lanceolate. .... Subgenus III. *Ceratocephalus.*
    - Perennial; hypocotyl not simulating a tap-root; fruit 1-chambered, utricular, the beak not lanceolate.
      - Pericarp plane, except for one longitudinal ribbed angle on each face; sepals thick, not marcescent in fruit; petals yellow; leaves simple, entire. Subgenus IV. *Oxygraphis.*
      - Pericarp smooth or sometimes with the lateral and distal portions reticulate; sepals thin, marcescent in fruit; petals red or rarely white; leaves compound, dissected into ligulate leaflets. Subgenus V. *Crymodes.*
- Achenes roughly transversely ridged; petals not glossy, white, the claws sometimes yellow; aquatic. .. .. Subgenus VI. *Batrachium.*

Sepals 3 or sometimes 4.
  Cotyledons 2; achenes constricted at the middles; style present, achene beaked; flowering stems arising from rhizomes.
    Portion of the achene distal to the constriction not filled with spongy parenchyma; receptacle greatly enlarged in fruit, depressed-globose; petals perhaps white, 5 or commonly 7–10; rhizomes and scapes markedly fistulous, 2–6 mm. in diameter; leaf-blades entire or 3-lobed or -parted; subaquatic. .... Subgenus VII. *Pallasiantha.*
    Portion of the achene distal to the constriction occupied by parenchymatous tissue; receptacle not much enlarged in fruit, subglobose; petals yellow, 5–6; rhizomes and scapes wiry, not fistulous, about 2 mm. in diameter; leaf-blades 3-parted and often again lobed or parted; terrestrial. Subgenus VIII. *Coptidium.*
  Cotyledon apparently 1, the other enclosed by it and undeveloped; achene not constricted, not containing spongy parenchyma; style wanting, achene beakless; flowering stems from a fascicle of fleshy roots, each of which produces an independent plant the following year; petals yellow. .... Subgenus IX. *Ficaria.*

SUBGENUS I. EURANUNCULUS (GREN. & GODR.) A. GRAY

KEY TO THE SECTIONS

Leaves (either the cauline or the basal) lobed, parted, or divided.
  Achenes smooth, sometimes hairy.
    Style and achene-beak present, the achene not corky-keeled or with corky thickening on the margin of the body; nectary-scale ventral to the nectary, covering it, apically truncate or rounded (the margins prolonged in *Ranunculus Eastwoodianus*, Section *Epirotes*).
      Nectary-scale free laterally for at least two-thirds its length, not forming a pocket (except in *R. recurvatus*); dorsoventral measurement of the achene 3–15 times the lateral; receptacle in fruit (in most species) 1–3 times the length in anthesis; sepals usually not lavender- or purple-tinged. .... Section 1. *Chrysanthe.*
      Nectary-scale attached to the petal laterally and forming a pocket; dorsoventral measurement of the achene 1–2.5 times the lateral; receptacle in fruit (in most species) 3–15 times the length in anthesis; sepals always tinged dorsally with purple or lavender. .... Section 3. *Epirotes.*
    Style and achene-beak practically lacking or if otherwise the achene with a corky keel or with corky thickening on the margin of the body; nectary-scale with the gland in a pocket on the ventral surface or the scale, forked and prolonged anteriorly on the surface of the petal or surrounding the gland; aquatic or palustrine. .... Section 5. *Hecatonia.*
  Achenes covered with spines, hooks, or papillae or with papillae produced into hooked hairs, rarely smooth; dorsoventral measurement of the achene 3–6 times the lateral; receptacle in fruit 1–3 times the length in anthesis. .... Section 2. *Echinella.*
Leaves (both cauline and basal) entire, dentate, serrulate, or wavy; dorsoventral measurement of the achene not more than twice or thrice the lateral. .... Section 4. *Flammula.*

SUBGENUS II. CYRTORHYNCHA (NUTT.) A. GRAY

KEY TO THE SECTIONS

Petals larger than the sepals.
- Fruiting receptacle enlarged to several times its size in anthesis, cylindrical or long-ovoid; nectary-scale overarching the nectary, truncate, the margins free from the blade of the petal; stolons present; leaves simple. .... Section 1. *Halodes.*
- Fruiting receptacle but slightly enlarged from its size in anthesis; nectary-scale not overhanging the nectary, consisting of a mere transverse ridge below the gland; stolons not present; leaves compound. .... Section 2. *Eucyrtorhyncha.*

Petals smaller than the sepals; nectary-scale forked, the lateral margins attached to the blade of the petal; stolons never present; receptacle but slightly enlarged in fruit, not cylindrical; leaves simple.
- Sepals yellow; blades of the petals 7–8 mm. long, yellow. ..... Section 3. *Arcteranthis.*
- Sepals white; blades of the petals 1–3 mm. long, yellowish or greenish. Section 4. *Pseudaphanostemma.*

Subgenera III–IX are composed each of a single section.

SUBGENUS II. CYRTORHYNCHA (NUTT.) A. GRAY
SECTION 1. HALODES (A. GRAY) L. BENSON

63. RANUNCULUS CYMBALARIA Pursh. Fl. Am. Sept. **2**: 392. 1814. *R. nanus* Fisch. in DC. Prodr. **1**: 33. 1824, as syn. *R. Cymbalariae* var. *americanus* DC. Prodr. **1**: 33. 1824. *Oxygraphis Cymbalaria* Prantl in Engl. Bot. Jahrb. **9**: 263. 1888. *Cyrtorhyncha Cymbalaria* Britt. Mem. Torrey Club **5**: 161. 1894. *Halerpestes Cymbalaria* Greene, Pittonia **4**: 208. 1900. *R. Cymbalaria* f. *hebecaulis* Fern. Rhodora **16**: 162. 1914.

Scapes erect, 2–5 or 11 cm. long, usually branched, stolons several dm. long, both about 1 mm. in diameter, not fistulous; radical leaf blades simple, cordate, ovate, or reniform, 5–22 mm. long, 4–20 mm. broad, crenate or sometimes merely 3-lobed at the apex in the same individual, proximally cordate or rounded and distally rounded, petioles 2–5 cm. long, stipular leaf bases 4–9 mm. long; sepals 3–5 mm. long; petals 3–5 mm. long; stamens usually 15–25; achenes 40–50 in a cylindrical head 3–8 or 13 mm. long, 3–4 or 6 mm. in diameter.

Mud of especially brackish streams and marshes; at least approached by forms in the Himalaya Mountains and Siberia; Alaska and the Yukon to Labrador and southward to Wyoming, Oklahoma (Harper County), Minnesota, Wisconsin, Illinois (Chicago), and New Jersey. Mostly coastal in New England. Northern coniferous, northeastern pine, and hardwood forests; prairie grassland. Late May to August.

Type collections: (1) *R. Cymbalaria*, "In saline marshes near the salt works of Onondago, New York." (2) *R. nanus*, none given. (3) Var. *americanus*, same as *R. Cymbalaria*, based upon a reference to it. (4) *F. hebecaulis*, "ALBERTA: Banff, alt. 4,500 ft., June 11, 1906, *Butters & Rosendahl*, no. 1339 (TYPE in Gray Herb.)"

63A. RANUNCULUS CYMBALARIA var. SAXIMONTANUS Fern. Rhodora **16**: 162. 1914. *R. tridentatus* H. B. K. var. *major* H. B. K. in DC. Syst. **1**: 253. 1818, not *R. Cymbalaria* var. *major* Hook. & Thomson in 1855.

Scapes 5–30 cm. long, usually branched; basal leaf blades cordate, ovate, or rarely reniform, 12–40 mm. long, 10–33 mm. broad, mostly crenate; sepals and petals 4–8 mm. long; stamens usually 20–35; achenes 100–300 in a head 5–12 mm. long, 3–5 mm. in diameter.

Mud of ocean inlets and of streams and marshes up to 2,300 meters elevation; Vancouver Island, British Columbia to Alberta and Nebraska (Hay Springs) and Kansas (Logan County); southward through the Great Basin to Baja California and Central Mexico; on the Pacific Slope occurring only about Puget Sound, along the lower Columbia River, in the mountains of Kern County, California, and in Southern California. Primarily Northern Desert and Plains Grassland; Oak Woodland and Southern Desert (along the Mojave River) in Southern California. Summer.

Type collections: (1) Var. *major,* "Juxta Carpio in Mexico." "Ad lacum S. Christobal alt., 1180 hex." (2) Var. *saximontanus.* Nomen novum for *R. tridentatus* var. *major.*

63B. RANUNCULUS CYMBALARIA var. ALPINA Hook. Fl. Bor. Am. **1**: 11. 1829.

Scapes 2–7 cm. long, usually unbranched; basal leaf blades trapezoidal to rectangular or some of them almost cordate, 4–10 mm. long, 2.5–8 mm. broad, 3-toothed at the apices and normally not crenate; sepals 2–3 mm. long; stamens about 10; achenes 25–60 in a head 3–5 mm. long by 3–4 mm. in diameter.

Mud; Siberia and the Himalaya Mountains, Asia; Alaska, Quebec, and Newfoundland; specimens from Wyoming appear also to belong to this variety (Snake and Lower Hoback Rivers and Upper Hoback Basin at 1,900–2,250 meters). Northern coniferous forest. Summer.

Numerous intermediate forms between this variety and the typical species occur from Quebec to Newfoundland and Nova Scotia and a similar type has been collected near Great Salt Pond, Rhode Island.

Type collection: "Found by Mr. Drummond upon the Rocky Mountains."

SECTION 2. EUCYRTORHYNCHA L. BENSON

64. RANUNCULUS RANUNCULINUS (Nutt.) Rydb. Bot. Surv. Nebr. **3**: 23. 1894. *Cyrtorhyncha ranunculina* Nutt. ex Torr. & Gray, Fl. N. Am. **1**: 26. 1838. *R. Nuttallii* A. Gray, Proc. Acad. Phila. **15**: 56. 1864. *Cyrtorhynca neglecta* Greene, Pittonia **4**: 146. 1900. *Cyrtorhyncha rupestris* Greene, Pl. Baker. **3**: 3. 1901. *R. neglectus* Wheeler, Rhodora **39**: 51. 1937.

Mountain-sides at 1,800–2,700 meters elevation; southeastern Wyoming and southward through middle Colorado to Sandoval County, New Mexico. Chiefly Western Pine forest. Mostly May and June.

The apetalous form (*Cyrtorhyncha neglecta* Greene), occurring near Golden and Morrison, Colorado, is poorly segregated. It is inconsistent in presence or absence of petals, and, since there seems to be no other character

to base the species upon, it is perhaps better neglected. Plants collected six miles southeast of Estes Park, *L. Benson 4942,* show reduction of petal number or absence of petals apparently in anthesis. However, some of the "flowers" have one or two pistils developed into achenes, and it is possible that others may have only abortive or unfertilized pistils past anthesis. *Cyrtorhyncha neglecta* was not based upon similar material, for even the flower buds show no petals in those individuals among the specimens which have apetalous flowers.

Type collections: (1) *C. ranunculina,* "By sides of gravelly brooks in the eastern Range of the Rocky Mountains, around the place known by the name of Independence Rock on the banks of the Sweet Water of the Platte, but not farther to the westward. Flowers in June." *Nuttall.* (2) *R. Nuttallii.* Nomen novum for *C. ranunculina.* (3) *C. neglecta,* "Occasional in dry ravines about Golden and Morrison, in middle Colorado, at about 6,000 feet altitude, flowering late in May. . . . I obtained it first in 1871, in ravines about Golden City. . . . Last spring I requested Mr. E. Bethel of Denver to go in search of it . . . with the result that I soon had fine flowering specimens of my long neglected plant, and later, some thoroughly mature achenes." The writer did not note the presence of Greene's 1871 collection in the Herbarium Greeneanum in 1935. However, it is represented in the New York Botanical Garden. (4) *C. rupestris,* "On moist cliffs in the Black Canon [Gunnison Watershed] in Colorado, 20 June, n. 198," *C. F. Baker* in 1901. Type, *HGr. 2269–70.*

SECTION 3. ARCTERANTHIS (GREENE) L. BENSON

65. RANUNCULUS COOLEYAE Vasey & Rose, Contr. U. S. Nat. Herb. **1**: 289. *pl. 22.* 1893. *Kumlienia Cooleyae* Greene, Erythea **2**: 193. 1894. *Arcteranthis Cooleyae* Greene, Pittonia **3**: 190. *pl. 3.* 1897.

Slopes near snow banks; Alaska to the mountains of western British Columbia; Colonel Bob Lookout, Olympic Mountains, Washington (*J. W. Thompson*). Northern coniferous forest. June to August.

Type collection: "Collected in fruit by Miss Grace Cooley, near the top of a snow-covered ridge, among loose rock, near Juneau, Alaska, August 6, 1891, and collected in flower by Mr. Frederick Funston, near the top of a bare mountain (1,000 meters high) of the St. Elias Alps, above Disenchantment Bay, Alaska, August 10, 1892." The Cooley specimen is designated as a LECTOTYPE.

SECTION 4. PSEUDAPHANOSTEMMA (A. GRAY) L. BENSON

66. RANUNCULUS HYSTRICULUS A. GRAY, Proc. Am. Acad. **7**: 328. 1867. *Kumlienia hystricula* Greene, Bull. Calif. Acad. **1**: 337. 1886.

Moist places near streams at 1,000–1,800 meters elevation; often about waterfalls and notably so at Yosemite Valley; Sierra Nevada from Butte County to Tulare County, California. Western pine forest. May and June.

Type collection: "Foot-hills of the Sierra Nevada, at Forest Hill and at New Castle, Placer Co., April, 1865, Bolander. Also, 1866, by Mr. Rattan, a state with longer petioles (6 inches long), and scapes nearly a foot high: apparently wet soil." The Bolander specimen from Forest Hill is designated as a LECTOTYPE.

SUBGENUS III. CERATOCEPHALUS (MOENCH) L. BENSON

67. RANUNCULUS TESTICULATUS Crantz, Stirp. Austr. Ed. 1. fasc. 2. 97. 1763.

Introduced from the Old World and now becoming common in the Intermountain Region from Eastern Washington and Eastern Oregon to Utah. April and May.

As pointed out to the writer by Dr. Ray J. Davis of the University of Idaho, Southern Branch, there are 1–3 ovules in a pistil. Study of the Old World plants may lead to elimination of this group from *Ranunculus.*

Type collection: Old World.

Specimens examined: WASHINGTON: Grand Coulee 8 miles north of the Dry Falls, *Rogers 287, 386, WSC, B.* OREGON: *Congdon,* Gilliam County, *J. W. Thompson 11347, T, GH, B.* IDAHO: S. E. of Pocatella, *R. J. Davis 1892, ISB, B.* UTAH: Mill Creek Canyon, Salt Lake City, *Garrett 6100, G, B.*

SUBGENUS IV. OXYGRAPHIS (BUNGE) L. BENSON

It is to be noted that the nectary scale of the subgenus *Oxygraphis,* as far as it has been studied, is a transverse ridge of type 1 (cf. L. Benson, Am. Jour. Bot. **27**: 799, 1940). This matter was not clear at the time of previous publication.

68. RANUNCULUS KAMCHATICUS DC. Syst. **1**: 302. 1817. *Ficaria glacialis* Fisch. ex DC. Sept. **1**: 305. 1817, not *Ranunculus glacialis* L. in 1753. *Oxygraphis glacialis* Bunge, Verz. Suppl. Fl. Alt. 35 (in reprint at Gray Herbarium). 1836.

Wet ground near sea level; Boreal regions and high mountains of Asia to Teller Ranger Station (Seward Peninsula) and Popof Island (Shumigan Islands), Alaska. Boreal and alpine tundra. Summer.

Type collection: (1) *R. kamchaticus,* "Hab. in Kamchatka. *h. Pall.* . . . (Vs. sp. in h. Pall, nunc. Lam.)" (2) *F. glacialis,* "Hab. in Daouria ad apicem montis Tchokondo. *Pansner.*"

SUBGENUS V. CRYMODES A. GRAY

The supposed very close relationship of this subgenus to the subgenus *Oxygraphis* is erroneous. The supposition was based upon overemphasis of one of the characters in common (persistent sepals) and upon ignoring of the numerous differences. Division of the genus *Ranunculus* would not permit inclusion of *Crymodes* under *Oxygraphis,* since it is not more closely related to that subgenus than to several others. Any segregation of this group of species should involve careful analysis of the characters of *R. juniperinus,* since its fruit is the most primitive found in any of the four.

Sepals densely reddish-brown-pilose or -hirsute; beak of the fruit 2–3 mm. long; flowering stems with 2 or more leaves; boreal and alpine.
Pericarp differentiated into a basal and central indurated portion around the seed and an apical and marginal reticulate scarious

portion; cauline and basal leaves large and triternately-compound; hairs not straight and arising singly. .... 69. *R. glacialis.*

Pericarp not markedly differentiated, the reticulations of the beak slightly more pronounced than those of the body; cauline and basal leaves 1–2 cm. long, 3-divided, but not compound; hairs straight, arising in clusters from papillae. .... 70. *R. Chamissonis.*

Sepals glabrous; beak of the fruit 0.5 mm. long; stems with not more than one small leaf; pericarp undifferentiated; sagebrush deserts or low mountains in the juniper-pinyon belt.

Fruits 4–6 mm. long; scapes with usually 1 bract and one or two flowers. 71. *R. juniperinus.*

Fruits 9–14 mm. long; scapes practically always with no bracts and with one flower. .... 72. *R. Andersonii.*

69. RANUNCULUS GLACIALIS L. Sp. Pl. 553. 1753. *Oxygraphis vulgaris* Freyn, Flora **70**: 141. 1887.

Wet ground, often near snow; Greenland and Iceland to Northern Europe and the Alps. Arctic-alpine tundra. Summer.

Type collection: "Habitat in Alpibus Lapponiae, Helvetiae."

70. RANUNCULUS CHAMISSONIS Schlect, Animad. Ranunc. **1**: 12. 1819.

Wet places; Nome and Cape Prince of Wales, Alaska, on Bering Strait. Boreal tundra. Collected by A. E. and R. T. Porsild, who kindly loaned the specimens in the National Herbarium of Canada to the University of Arizona. August.

Type collection: "Habitat ad pedes montium glacialium loca uda glacie deliquescente madida in sinu St. Laurentii, qui in littore Asiae arcticae orientalis, haud procul a freto Behringii meridiem versus est situs, unde magnam hujus plantae copiam attulit amicissimus de Chamisso in cujus honorem hanc pulchram dixi speciem."

71. RANUNCULUS JUNIPERINUS Jones, Proc. Calif. Acad. II. **5**: 616. 1896. *R. Andersonii* A. Gray var. *tenellus* S. Wats. King's Rept. **5**: 7. 1871. *Beckwithia juniperina* Heller, Muhlenbergia **1**: 144. 1906.

Rocky slopes at 1,600–2,000 meters elevation; Karshaw, Meadow Valley Wash, Eastern Nevada; Utah; Virgin Mountains, Arizona. Southwestern coniferous woodland. May.

This is the least specialized of the North American species in this subgenus, at least so far as fruit is concerned.

Type collections: (1) Var. *tenellus*, "Pilot Rock Point, Salt Lake, Utah. (17.)" *Sereno Watson*, June 17, 1869, 5,000 feet altitude. (2) *R. juniperinus*, "No. 5011, April 4, at Copper Mine, 18 miles west of St. George, Utah, in Beaverdam Mts., 5000° alt., among junipers in loose gravelly soil. No. 5139, April 30, at head of the west branch of Santa Clara Valley in the Beaverdam Mts., Utah, 5000° alt., in loose soil on rocks, among junipers." *Jones 5011* is designated as a LECTOTYPE. Herbarium of Pomona College.

72. RANUNCULUS ANDERSONII A. Gray, Proc. Amer. Acad. **7**: 327. 1867. *Oxygraphis Andersonii* Freyn, Flora **70**: 140. 1887. *Beckwithia Austinae* Jepson, Erythea **6**: 97. *pl. 1.* 1898. *Beckwithia Andersonii* Jepson, Erythea **6**: 99. 1898.

Rocky mountainsides at 1,000–1,500 meters elevation; southeastern Oregon to Ketchum, southcentral Idaho, and southward east of the Sierra Nevada to the Panamint Mountains, California, and to Nye County, Nevada. Northern desert. May.

Type collections: (1) "Near snow, on Blind Springs Mountain in the Eastern Sierra Nevada, Dr. C. L. Anderson, 1866." The specimen in the Gray Herbarium was collected near Carson City, Nevada, by Anderson in 1866. (2) *B. Austinae,* "Decomposed lava on the western treeless slope of Lake City Pass, Modoc County, California, June 1898." *Austin & Bruce 2134.*

SUBGENUS VI. BATRACHIUM (DC.) A. GRAY

The treatment of the subgenus *Batrachium* here differs from that of W. B. Drew, Rhodora **38**: 1–47. 1936, chiefly in the broader interpretation of species and varieties. The categories recognized are nearly all the same, but several are accorded lower rank either as varieties or unnamed forms. Consequently, *R. trichophyllus* appears as *Ranunculus aquatilis* var. *capillaceus* based upon the same type, and *R. longirostris* appears as *R. circinatus,* a name first applied to an Old World type. The group is a complex one capable of nearly as many interpretations as there are botanists.

TYPE SPECIES, *Ranunculus hederaceus* L.

Receptacle glabrous.
  Plant with some non-dissected floating leaves.
    Style in anthesis half as long as the ovary; leaves all shallowly-lobed, none finely-dissected. . . . 72. *R. hederaceus.*
    Style in anthesis 2–3 times as long as the ovary; leaves dimorphous, the upper ones floating and deeply 3-parted, the lower ones submersed and dissected into filiform divisions. 73. *R. Lobbii.*
  Plant with no non-dissected floating leaves. . . . 74D. *R. aquatilis* var.
Receptacle hispid; style in anthesis not longer than the ovary.
    Achene beaks 0.2–0.5 mm. long. . . . 74. *R. aquatilis* vars.
    Achene beaks 0.7–1.1 mm. long. . . . 75. *R. circinatus.*

72. RANUNCULUS HEDERACEUS L. Sp. Pl. 556. 1753. *Batrachium hederaceum* S. F. Gray, Nat. Arr. Brit. Pl. **2**: 271. 1821.

Aquatic in fresh water at low elevations; Europe; native or perhaps naturalized in Newfoundland and along the coastal plain in Chester County, Pennsylvania, along Chesapeake Bay, and (according to report) at Charleston, South Carolina (cf. W. B. Drew, Rhodora **38**: 5–7. 1936). Late April to early summer.

Type collection: "Habitat in aquis vadosis, Angliae, Belgii." *R. hederaceus* L. var. *hederaefolius* Lawson, Trans. Roy. Soc. Canada 2(4): 44–45. 1884, was based upon an Old World type, *R. hederaefolius* Salisb. Prodr. Stirp. 373. 1796.

73. RANUNCULUS LOBBII (Hiern) A. Gray, Proc. Am. Acad. **21**: 364. 1886. *R. Hydrocharis* Spenn. f. *Lobbii* Hiern, Jour. Bot. Brit. & For. **2**:

66. *pl. 14.* 1871. *R. hederaceus* var. *Lobbii.* Brew. & Wats. Bot. Calif. **1**: 5. 1876. *R. aquatilis* L. var. *Lobbii* S. Wats. Bibl. Ind. N. Amer. Bot. **1**: 17. 1878. *Batrachium Lobbii* Howell, Fl. N. W. Am. **1**: 13. 1897.

Shallow vernal pools near sea level; Vancouver Island, British Columbia; Corvallis, Oregon; California from Sonoma and Lake counties and the Vaca Mountains to the Santa Cruz Mountains and Alameda County. Northwestern coniferous forest and oak woodland. Late February to early May.

Type collection: "Oregon, *W. Lobb.* 1852. *n. 249.*"

74. Ranunculus aquatilis L. Sp. Pl. 556. 1753.

This polymorphic Old World species is represented in North America by four varieties, as follows:

KEY TO THE VARIETIES

Receptacle densely hairy, the hairs usually more or less tufted.
  Uppermost leaves filiform-dissected and submersed like the lower ones.
    Stems 1–2.5 mm. in diameter; stamens usually 10 or more. 73 A. Var. *capillaceus.*
    Stems 0.4–1 mm. in diameter; stamens 5–8. .. .. 73 B. Var. *eradicatus.*
  Uppermost leaves (1–10 of them) not filiform-dissected, merely lobed, parted, or divided, floating. . . . 73 C. Var. *hispidulus.*
Receptacle glabrous or sparsely hairy and the hairs not tufted. . 73 D. Var. *calvescens.*

74A. Ranunculus aquatilis L. var. capillaceus (Thuill.) DC. Prodr. **1**: 26. 1824. *R. trichophyllus* Chaix, in Vill. Hist. Pl. Dauph. **1**: 335. 1786. *R. capillaceus* Thuill. Fl. Par. Ed. 2. **1**: 278. 1799. *R. amphibius* James, Long Exped. Rocky Mts. **1**: 498. 1823. *R. aquatilis* L. var. *brachypus* Hook. & Arn. Bot. Beech. Voy. 316. 1840. *Batrachium trichophyllum* F. Schultz, Arch. Fl. France et All. **1**: 107. 1848. *R. aquatilis* var. *trichophyllus* A. Gray, Man. Bot. Ed. 5. 40. 1867. *R. hydrocharis* Spenn. f. *trichophyllus* Hiern. Jour. Bot. Brit. & For. **9**: 101. 1871. *R. Porteri* Britt. Bull. Torrey Club **17**: 310. 1890. *Batrachium Bakeri* Greene ex Baker, W. Amer. Pl. **1**: 7. 1902, *nomen nudum.* *Batrachium Bakeri* Greene, Leafl. Bot. Obs. & Crit. **1**: 95. 1904. *Batrachium pedunculare* Greene, Leafl. Bot. Obs. & Crit. **1**: 95. 1904. *Batrachium aquatile* (L.) Wimm. var. *trichophyllum* Frye & Rigg. N. W. Fl. 169. 1912. *Batrachium aquatile capillaceum* Garrett, Spring Fl. Wasatch Reg. Ed. 3. 35. 1917. *Batrachium Porteri* Britt. in Rydb. Fl. Rocky Mts. & Adj. Plains 294. 1917. *R. aquatilis* var. *Bakeri* Jepson, Fl. Calif. **1**: 544. 1932. *R. aquatilis* var. *peduncularis* Jepson, Fl. Calif. **1**: 544. 1922. *R. subrigidus* W. Drew, Rhodora **38**: 39. 1936.

Glabrous or hispidulous aquatic perennials; roots 0.3–0.4 mm. in diameter; stems submersed, rooting adventitiously at the lowest nodes, 2–6 or rarely 20 dm. long and 1–2.5 mm. in diameter, branching, with large air chambers present in the cortex, the vascular system far in the interior, surface sometimes hispidulous; leaf blades all submersed and finely dissected into filiform divisions, usually but not necessarily collapsing when withdrawn from the water, 2–4 cm. long, 3–5 cm. broad; petioles 0.2–3 cm. long, including the stipular leaf bases which are 2–5 mm. long (these often bordering the entire petiole and sometimes apically free from it) cauline leaves alternate; pedicels stout, 1–2 cm. long in flower and 1.5–3 cm. long in fruit,

glabrous; sepals 5, light green, spreading, ovate, 2–3 cm. long, 1–1.8 mm. broad, glabrous, half the length of the petals, deciduous before the corolla; petals 5, white or the bases yellow, 4–8 mm. long, 1.5–2 mm. broad, the nectary scale glabrous, forming a shallow pocket or sometimes greatly reduced; stamens 5 or 10–25; achenes usually 10–20 in a globose cluster, each achene obovoid, 1–1.5 mm. long or rarely longer, 1–1.5 mm. dorsoventrally, 0.5–0.7 mm. laterally, roughly transversely-rigid, glabrous from the beginning or the pistils hispid and the achenes glabrate or with some hairs persisting on or near the dorsal sutures, margin rather sharp, the achene beak about 0.3 or rarely 0.5 mm. long; receptacle subglobose, 1 mm. long in flower and 1 mm. long in fruit, densely pubescent.

Ponds and ditches and vernal streams and pools at low elevations along the coast and up to 1,000 or 1,800 meters elevation in the interior; Europe; Asia; Alaska to Labrador and southward to California (not in the humid coast forest), northern Mexico, Kansas, Kentucky, and the vicinity of New York City. Rare in New England. Spring or summer.

The form with hairy pistils and glabrate or partly-hairy achenes occurs from Bering Strait, the Pribilof Islands, and the Yukon River watershed southward through the northern Great Basin and Great Plains to southeastern California, northern Mexico, and Nebraska and eastward mostly north of the Canadian Boundary to Newfoundland, Vermont, and Maine. This form and the one with glabrous pistils occur together through large areas, but each has a large territory wherein it is the only form present. A robust form with achenes 2–2.5 mm. long occurs in Eastern Oregon and extreme northern California. The following specimens of this form are at the Gray Herbarium: OREGON: Prairie City, *Henderson 5448;* Shirk, *Leiberg 2586.* CALIFORNIA: Buck Mountain, Humboldt County, California, *Harris, Tracy and Yates 3453;* Hat Creek, Shasta County, California, *Eggleston 7521.*

Type collections: (1) *R. trichophyllus,* "trichophyllus (mihi) Hall. 1162: in rivulis limpidis, Valgaud. Devoluy." For valid publication, the species depends upon the reference to Haller's *1162,* "RANUNCULUS caule fluitante, petiolis uniflorus, foliis capillaribus, laciniis divergentibus," for Chaix gave no description of the plant. Haller's references are capable of several interpretations, and there has been much confusion about them. However, as pointed out by Drew, Rhodora **38**: 21–23. 1936, the common usage has been to attribute the name *Ranunculus trichophyllus* to the common dissected-leaved plant of Switzerland and much of Europe. (2) *R. capillaceus,* based upon Haller *1162,* and therefore upon the same type as *R. trichophyllus.* (3) *R. amphibius,* Platte River, west of the mouth of Portera's Creek. This plant is the form with hairy pistils discussed above. A specimen is in the New York Botanical Garden. (4) Var. *brachypus,* "California-Supplement. Where not otherwise mentioned, it is to be understood that the following species are from the collection of Mr. Douglas. They were presented by the Horticultural Society of London, in whose service Mr. Douglas was at the time he gathered them." (5) *R. Porteri,* "Henry's Fork,

No. 1062; *Ranunculus,* entirely immersed." Eastern Idaho, according to Rydberg; Southwestern Wyoming, according to Britton. (6) *B. Bakeri,* "Pools among the hills of the Coast Range near Stanford University California, 8 May, 1902, C. F. Baker, distributed under n. 786." Type seen at Notre Dame, but *Hgr. No.* not recorded. (7) *B. pedunculare,* Near Lakeport, Lake Co. [California], 9 May, 1902, C. F. Baker, distributed under n. 3062." Type seen at Notre Dame, but *HGr. No.* not recorded. (8) *R. subrigidus,* "QUEBEC. . . . York River, July 29, 1905, *Williams, Collins, & Fernald* (TYPE in Gray Herb.). In addition to the names given in synonymy various new combinations of Old World names have been made in America (*Batrachium aquatile pantothrix* Piper Contr. U. S. Nat. Herb. **11**: 270. 1906, based upon *R. pantothrix* Brot. Fl. Lusit. **2**: 375. 1804; *Batrachium aquatile flaccidum* Cockerell in Daniels Fl. Boulder, Colo., 122. 1911 and *R. aquatilis* L. var. *flaccidus* Rob. in A. Gray, Syn. Fl. **1**: 21. 1895, based upon *R. flaccidus* Pers. in Usteri, Ann. Bot. **5**(14): 39. 1795; *B. aquatile caespitosum* Piper, Contr. U. S. Nat. Herb. **11**: 270. 1906 and *B. trichophyllum caespitosus* Britt. & Brown, Ill. Fl. **2**: 84. 1897, based upon *R. caespitosus* Thuill. Fl. Env. Paris Ed. 2. 278. 1799; *R. aquatilis* L. var. *Drouetii* Lawson, Trans. Roy. Soc. Can. 2: 45. 1884, based upon *R. Drouetii* F. Schultz, Arch. Fl. France et All. 1: 107. 1848). *R. divaricatus* Schrank has been used also under various combinations for this plant.

R. amphibius James (*R. subrigidus* Drew) is intermediate between *R. aquatilis* var. *capillaceus* and *R. circinatus.*

74B. RANUNCULUS AQUATILIS var. ERADICATUS Laestad. N. Act. Reg. Soc. Scient. Ups. **11**: 242. 1839. *Batrachium eradicatum* Fries, Bot. Notis. 114. 1843, *nomen nudum. B. confervoides* Fries, Bot. Notis. 121. 1845. *R. confervoides* Fries, Summa Veg. Scand. **1**: 139. 1845. *R. paucistamineus* Tausch var. *borealis* Beurl. Bot. Notis. 156. 1852. *R. hydrocharis* Spenn. f. *confervoides* Hiern, Jour. Bot. Brit. & For. **9**: 102. 1871. *R. aquatilis* var. *confervoides* Lawson, Trans. Roy. Soc. Canada **2**(4): 45. 1884. *B. paucistomineum* (Tausch.) F. Schultz var. *eradicatum* Gelert. Bot. Tidsskr. **19**: 28. 1894. *R. aquatilis* L. var. *confervoides* Rob. in A. Gray Syn. Fl. **1**: 21. 1895. *R. divaricatus* Schrank var. *eradicatus* Williams Jour. Bot. Brit. & For. **46**: 21. 1908. *R. flaccidus* Pers. var. *confervoides* Hegi. Ill. Fl. Mittel-Eur. **3**: 584. *f. 708k.* 1912. *B. confervoides* Rydb. Fl. Rocky Mts. & Adj. Plains 294. 1917. *R. paucistamineus* var. *eradicatus* (Laest.) Gelert *f. terrestris* Porsild, Meddel. Grnl. **58**: 77. 1926. *R. trichophyllus* Chaix var. *eradicatus* W. Drew, Rhodora **38**: 33. 1936.

Stems 0.4–1 mm. in diameter; leaves all submersed and dissected into divisions 0.1 mm. broad and 3–4 cm. long; petals 4–6 mm. long by 2 mm. broad; stamens 5–8; achenes 1 mm. long, glabrous from the beginning.

Stagnant water; circumboreal; Arctic America in Alaska and the Yukon District and from Quebec to Labrador and Newfoundland; Greenland. A modified form continues southward into New England. Arctic-alpine grassland and northern coniferous forest. July and August.

Type collections: (1) Var. *eradicatus.* The following information was supplied by Dr. H. W. Rickett of the New York Botanical Garden: "No specimen or collection mentioned. Habitat in shallow pools, and L. thought it originated by action of ice on plant of typical *R. aquatilis.* Various locations

in Torne Lappmark are given as examples." The following is quoted from W. B. Drew, Rhodora **38**: 45. 1936, "It is quite evident from Laestadius's remarks that he believed the filiform and diminutive habit of the plants to be due to ice-action in pulling up the roots so that the new growth arose from fragments of the old. In other words, he regarded var. *eradicatus* as simply a variation, brought about by the rigors of a subarctic climate, of the usual dissected-leaved European *R. aquatilis.*" (2) *R. confervoides, "In aquosis* Lapponiae *et* Finlandiae *borealis.* v. c. ad Ulaeborg, unde specimina a A. Nylander missa dedit Angstrom." TYPE in the University Museum, Upsala. (3) Var. *borealis,* "R. aquatilis eradicatus Laest. Batrachium aquatile eradicatum Fr. Mant. 3. B. confervoides Fr. in bot. notis, Herb. norm. fasc. 13. n: ro 45." (4) *F. terrestris,* "At the border of a little lake in Blaesdalen 69° 17 Disko . . . was just flowering Aug. 1913 (Th. P. !)" *Thorbjørn Porsild.* Disko Island, Greenland.

74C. RANUNCULUS AQUATILIS var. HISPIDULUS E. Drew, Bull. Torrey Club **16**: 150. 1889. *R. aquatilis* L. "form" *heterophyllus* A. Gray, Proc. Am. Acad. **21**: 363. 1886. *R. Grayanus* Freyn, Deuts. Bot. Monatss. **8**: 179. 1890. *Batrachium Grayanum* Rydb. Fl. Rocky Mts. & Adj. Plains 294. 1917. *R. trichophyllus* var. *hispidulus* W. Drew, Rhodora **38**: 29. 1936.

Upper portion of the stem floating; floating leaves 1–10, simple, reniform, 0.5–1 cm. long, 1–3 cm. broad, 3-lobed or deeply-parted, the lobes again forked or parted, the ultimate lobes rounded, the leaf proximally cordate and distally rounded, sometimes hispidulous dorsally; petals 4–6 mm. long.

Ponds, ditches, and vernal streams and pools at low elevations along the coast and up to 1,000 or 1,800 meters elevation in the interior; Pacific Slope from the Shumigan Islands, Alaska, to Monterey and Mariposa Counties, California; Cuyamaca Lake, San Diego County, California; occasional in Eastern Washington and Oregon and rare in Idaho, Montana, and Utah. Practically confined to the northwestern coniferous forest from which var. *capillaceus* is almost excluded April to July.

Type collections: (1) "Form" *heterophyllus,* "The type of Linnaeus *R. aquatilis* being the form heterophyllus." The Pacific Coast plant was taken by Gray to be typical *R. aquatilis.* (2) Var. *hispidulus,* Jarnigan's, Humboldt County, *Chestnut & Drew,* July 21, 1888. (3) *R. Grayanus, "British Columbia.* Teich bei Lytton, auf Granit in 80–100 m. See hohe. . . . July 1887 (No. 331 p. p.)"

74D. RANUNCULUS AQUATILIS var. **calvescens** (W. Drew) L. Benson, comb. nov. *R. trichophyllus* Chaix var. *calvescens* W. Drew, Rhodora **38**: 32. 1936.

Achenes 1.5–1.8 mm. long, usually glabrous; receptacle glabrous or sparsely hairy, the hairs when present not densely-tufted; otherwise like var. *capillaceus.*

New Brunswick, Nova Scotia and New England; Cohasset, Herkimer County, New York; Keneewaw County, Michigan. Northern coniferous and northeastern hardwood forests. June to August.

Type collection: "MASSACHUSETTS: Cram's River, Danvers, July 2, 1885, *J. H. Sears.* (Type in Gray Herb.)"

75. RANUNCULUS CIRCINATUS Sibth. Fl. Oxon. 175. 1794. *Batrachium circinatum* Spach. Hist. Nat. Veg. **7**: 201. 1839. *R. longirostris* Godr. Mem. Roy. Soc. Nancy 39. *f. 9.* 1839. *B. longirostre* F. Schultz, Arch. Fl. France et All. **1**: 71. 1842. *R. hydrocharis* Spenn. f. *longirostris* Hiern. Jour. Bot. Brit. & For. **9**: 100. 1871. *R. aquatilis* L. var. *longirostris* Lawson, Trans. Roy. Soc. Can. **2**: 45. 1884. *R. usneoides* Greene, Leafl. Bot. Obs. & Crit. **2**: 106. 1910. *B. circinatum terrestre* Lunell, Am. Midl. Nat. **4**: 359. 1916.

Aquatic in sluggish fresh water; Old World; Nevada and Montana to western Quebec, New Mexico, Arkansas, Tennessee, New Jersey, and Delaware; rare in New England. Summer.

This species is connected with *R. aquatilis* var. *capillaceus* through a great many intergrading forms; *R. amphibius* James (*R. subrigidus* Drew). In works by American authors the following names based upon Old World types have been applied in various combinations: *Ranunculus divaricatus* Schrank; *Ranunculus stagnatilis* Wallr.; *Ranunculus rigidus* Roth.

Type collections: (1) *R. circinatus*, "Christ-Church Meadow—South Leigh." England. (2) *R. longirostris*, "In aquis fluentibus Americae Borealis prope Saint Louis, Missouri." *N. Riehl*, June, 1838. (3) *R. usneoides*, "Lake City, Arkansas, collected by Mr. A. H. Howell, 1 May, 1910." (4) *B. circinatum terrestre*, "A form growing on low land where water once was, but later dried up. Leeds." North Dakota, *Lunell*.

SUBGENUS VII. PALLASIANTHA L. BENSON

76. RANUNCULUS PALLASII Schlect. Animad. Ranunc. **1**: 15. *pl. 2.* 1820.

Shallow water near sea level; Bering Sea region and northern Alaska; eastern shore of Hudson Bay, lat. 60° 42′ and Port Harrison, Quebec. Arctic-alpine grassland. July and August.

Type collection: "Habitat in littore occidentali Americae summae arcticae in sinubus scilicet Eschscholtzii et bonae spei inque insula St. Georgii parva insula ultra insulas Aleuticas septentrionem versus sita (de Chamisso)."

SUBGENUS VIII. COPTIDIUM (NYM.) L. BENSON

77. RANUNCULUS LAPPONICUS L. Sp. Pl. 553. 1753. *Coptidium lapponicum* Beurl. in Gand. Fl. Eur. 234. 1883. *Anemone nudicaulis* A. Gray, Bot. Gaz. **11**: 17. 1886.

Bogs, at low elevations; Europe; Asia; Alaska to Labrador and Greenland and south to northern Minnesota, Ontario, and Maine (Aroostock River). Arctic-alpine grassland and northern coniferous forest. Summer.

Type collections: (1) *R. lapponicus*, "*Habitat in Alpibus* Lapponicus." (2) *A. nudicaulis*, "Which grows in bogs and on banks near the water at Sand Bay [Lake Superior region], Minnesota, very near lat. 48°, and in or near the Canadian Boundary . . . specimen sent to me in a letter, dated August 8, 1870, from Mr. Joseph C. Jones, then of the U. S. Steamer Search."

SUBGENUS IX. FICARIA (HUDS.) L. BENSON

78. RANUNCULUS FICARIA L. Sp. Pl. 550. 1753. *Ficaria verna* Huds. Fl. Angl. Ed. 1. 214. 1762. *Ficaria ranunculoides* Moench, Meth. 215. 1744. *Ficaria Ficaria* Karst. Pharm. Med. Bot. 565. 1880.

Moist land at low elevations; commonly a pasture weed; Europe; introduced along the coast in Massachusetts, on Long Island, in eastern Pennsylvania, at Washington, D. C., and in adjacent Maryland. April and May.

Type collections: (1) *R. Ficaria, "Habitat in Europae ruderalis, umbrosis, spongiosis."* (2) *F. verna,* based upon *R. Ficaria.* (3) *F. ranunculoides,* based upon *R. Ficaria.*

DEPARTMENT OF BOTANY, UNIVERSITY OF ARIZONA
TUCSON, ARIZONA.

BULLETIN OF THE TORREY BOTANICAL CLUB

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UNDESCRIBED PLANTS FROM MEXICO AND CENTRAL AMERICA[1]

C. L. LUNDELL

From the Yucatan Peninsula and Chiapas eighteen novelties are described in twelve families. Of particular interest are two new trees in *Symplococarpon*, one from British Honduras and the other from Chiapas; this genus of the Theaceae, proposed in 1937, now has eight representatives in Mexico and Central America. Two species, described in the subgenera *Chamaecrista* and *Peiranisia*, are transferred to *Cassia;* a slender vine, *Bradburya unifoliata* Rose, recently found in the Yucatan Peninsula, is placed in *Centrosema*, a conserved genus. Along with a record of *Ammannia Koehnei* Britton from Yucatan, *A. dentifera* A. Gray is relegated to a varietal status under *Rotala ramosior* (L.) Koehne.

Celtis petenensis Lundell, sp. nov. Arbor parva, ramulis pilosis. Folia petiolata, membranacea, ovata vel ovato-lanceolata, 2.5–8.5 cm. longa, 1.6–4 cm. lata, apice acuminata, basi acuta vel rotundata, serrata, trinervia, utrinque parce pilosa. Inflorescentiae cymosae, axillares, ad 1 cm. longae, pilosae. Fructus ellipsoideus.

A slender tree, 6–10 m. high, branchlets very slender, short pilose, the hairs incurved. Petioles slender, canaliculate, pilose, 3–7 mm. long. Leaf blades thinly membranaceous, dark green above, paler beneath, ovate or ovate-lanceolate, 2.5–8.5 cm. long, 1.6–4 cm. wide, apex acuminate, base oblique, acute or rounded, sometimes rounded and abruptly acutish, sharply serrate except at base, trinerved, the veins impressed above, conspicuous beneath, sparsely but persistently pilose on both surfaces, the hairs coarsest above, densest along the primary veins. Inflorescence cymose, up to 1 cm. long, the peduncle scarcely evident, apparently bearing hermaphrodite flowers at apex of cyme, the male below. Fruiting pedicels nodose, subequaling to slightly longer than petioles, very slender, short pilose. Drupes ellipsoid, with a ring of hairs at base and a few hairs at apex around the stigma, otherwise glabrous.

GUATEMALA—PETÉN: Lake Yaxha, on ruins of Topoxté, in *ramonal,* June 19, 1933, *C. L. Lundell 4306* (TYPE in the University of Michigan Herbarium), a slender tree, 6 to 10 m. high.

C. petenensis is allied to *C. Swartzii* Planch. of the West Indies, a tree with glabrous leaves. The species has been reported as *C. trinervia* Lam. (Lundell, Carnegie Inst. Wash., Publ. 478: 58. 1937). A sterile specimen, *Bartlett 12574* from Uaxactun, probably is referable here.

[1] Papers from the University of Michigan Herbarium. The study has been supported in part by funds from the Horace H. Rackham School of Graduate Studies of the University of Michigan.

Neea hirtella Lundell, sp. nov. Frutex, ramulis puberulo-hirtellis. Folia puberulo-hirtella, petiolata, chartacea, elliptica vel oblongo-elliptica, 3–8 cm. longa, 1.5–4.2 cm. lata, apice subabrupte subacuminata, basi acuta vel rotundata. Flores feminei cymoso-paniculati, paniculis parvis, rufo-puberulis, floribus sessilibus vel pedicellatis, pedicellis ad 2.5 mm. longis. Perianthium tubulosum, ca. 3 mm. longum, rufo-puberulum. Staminodia 7.

A shrub, about 1 m. high; branches terete, persistently short hirtellous; branchlets rather slender, drying blackish, usually with short internodes, rufo-hirtellous, the hairs incurved. Petioles short hirtellous, 2.5–11 mm. long. Leaf blades yellowish-green, sublucid on upper surface, slightly paler beneath, chartaceous, persistently short hirtellous on both surfaces or subglabrescent above with age, elliptic or oblong-elliptic, 3–8 cm. long, 1.5–4.2 cm. wide, apex subabruptly short acuminate, the acumen acute or obtusish, base acute or rounded, costa plane or nearly plane above, prominent beneath, primary veins 6 to 8 on each side, slender, veinlets inconspicuously reticulate. Pistillate inflorescence rufo-puberulent, small; the peduncle slender, up to 5 cm. long (in fruit). Pistillate flowers rufo-puberulent, sessile or borne on pedicels up to 2.5 mm. long, subtended by 3 linear-lanceolate bractlets about 1 mm. long; perianth tubular, scarcely 3 mm. long, 5-dentate. Staminodia 7. Ovary 1-celled with 1 erect ovule; stigma penicillate. Young fruits ellipsoid, about 9 mm. long, sparsely rufo-puberulent, dark red.

MEXICO—YUCATAN: Chichen Itza, in low thicket between the Castillo and Sacred Cenote, June 6, 1938, *C. L. Lundell & Amelia A. Lundell 7422* (TYPE in the University of Michigan Herbarium), a shrub, about 1 m. high, fruits ellipsoid, dark red; same locality, in forest, June 15, 1932, *W. C. Steere 1307*.

N. hirtella is characterized primarily by the minute but persistent pubescence. Its affinity is with *N. choriophylla* Standl., described from the same region.

Ocotea Matudai Lundell, sp. nov. Arbor, ramulis glabris, subatris. Folia petiolata, subcoriacea, lanceolata vel oblongo-lanceolata, 7–15 cm. longa, 2.6–5.5 cm. lata, apice obtusa, basi acuta, reticulata, supra glabra, lucida, subtus (axillis barbatis exceptis) glabra. Inflorescentiae cormyboso-paniculatae, puberulae, ad 7 cm. longae. Flores hermaphroditi. Filamenta antheras breviora. Antherae parvae, truncatae. Staminodia stipitiformia, glabra. Ovarium glabrum.

A tree, 7 m. high, branchlets slender, angled at first, glabrous. Petioles glabrous, canaliculate, 8–15 mm. long. Leaf blades subcoriaceous, shining, paler beneath, lanceolate or oblong-lanceolate, 7–15 cm. long, 2.6–5.5 cm. wide, apex obtuse, base acute, costa plane above, prominent beneath, primary veins 6 to 9 on each side, prominulous on both surfaces, veinlets reticulate, barbate beneath in the axils of the primary veins, glabrous otherwise. Inflorescences corymbose-paniculate, branched to the base, up to 7 cm. long, puberulent. Flowers perfect, about 7 mm. in diam., white, puberulent; pedicels slender, up to 4 mm. long. Perianth tube scarcely evident; lobes elliptic-oblong, about 2.5 mm. long, obtuse, puberulent on both surfaces. Receptacles pubescent. Stamens 9, not over 1.3 mm. long; filaments slightly shorter than anthers, glabrous; anthers 4-celled, ovate-oblong, apex truncate

and very shallowly emarginate, base sharply contracted into filament, cells of series I and II introrse; glands of series III rather small, sessile. Staminodia stipitiform, glabrous. Ovary glabrous, equaling style.

MEXICO—CHIAPAS: Mt. Ovando, western slope, alt. 1800 m., July 1–16, 1940, *Eizi Matuda 4221* (TYPE in the University of Michigan Herbarium); same locality, north slope, alt. 1300 m., *Matuda 4188*.

Capparis yucatanensis Lundell, sp. nov. Frutex, ramulis stellato-tomentellis, vinaceis, angulatis. Folia petiolata, chartacea, supra lucida glabra, subtus albido-stellato-tomentella, areolata, obovata, obovato-elliptica, vel elliptica, 3–6 cm. longa, 2.2–4.3 cm. lata, apice abrupte acuminata vel acuta, basi obtusa. Inflorescentiae umbellatae, supra-axillares, pedunculatae. Pedicelli tomentelli, ad 6 mm. longi. Fructus oblongo-ellipsoideus, albido-stellato-pubescentibus, stipitatus.

An arborescent shrub, about 4 m. high; branchlets usually short, angulate, slender, at first vinaceous tomentellous with minute stellate hairs. Petioles canaliculate, whitish tomentellous, 4–10 mm. long. Leaf blades chartaceous, lucid and glabrous above, white tomentellous beneath with soft minute stellate hairs, obovate, obovate-elliptic, or elliptic, 3–6 cm. long, 2.2–4.3 cm. wide, apex abruptly short acuminate or acute, base obtuse, costa and veins plane above, conspicuous beneath, primary veins 3 to 5 on each side, areolate beneath, the veinlets minutely and conspicuously reticulate. Inflorescence umbellate, solitary, supra-axillary, with only one flower developing; peduncles tomentellous, 4–7 mm. long; pedicels tomentellous, those of fruits rather stout, up to 6 mm. long. Calyx tomentellous, the calyx lobes 1-seriate. Fruits oblong-ellipsoid, white tomentellous, borne on stipes up to 4 mm. long.

MEXICO—YUCATAN: Chichen Itza, off Kaua road in advanced deciduous forest, June 8, 1938, *C. L. Lundell & Amelia A. Lundell 7452* (TYPE in the University of Michigan Herbarium), an arborescent shrub, about 4 m. high.

C. yucatanensis has affinity to *C. incana* H.B.K. The plant is characterized by its predominantly obovate leaves conspicuously reticulate veined beneath.

Crataeva glauca Lundell, sp. nov. Arbor parva, glabra. Folia trifoliolata, petiolata; foliola membranacea, glauca, elliptica, oblongo-elliptica, lanceolata, vel obovata, 3.5–10.5 cm. longa, 1.7–4.8 cm. lata, apice subabrupte acuminata, basi cuneata vel acutiuscula. Inflorescentiae terminales, corymbosae. Sepala 4, lineari-lanceolata, 4–7 mm. longa. Petala 4, lamina anguste oblongo-elliptica, 2.3–5 mm. lata, ad 12 mm. longa. Fructus globosus, ad 3.8 cm. diam.

A small tree, up to 7 m. high, 30 cm. in diam., glabrous; branchlets rather stout, with conspicuous scattered lenticels. Leaves 3-foliolate; petioles slender, up to 9.5 cm. long; petiolules short, usually 1.5 to 3 mm. long, rarely as much as 6.5 mm. long. Leaflets membranaceous, glaucous beneath, elliptic, oblong-elliptic, lanceolate, or the terminal leaflet sometimes obovate, usually 3.5 to 7.5 cm. long, sometimes up to 10.5 cm. long, 1.7 to 4.8 cm. wide, apex subabruptly acuminate, the acumen usually short, terminal with cuneate base, lateral unequal-sided and acutish, primary veins 6 or 7 on each side, prominulous beneath. Inflorescence a terminal leafy corymbose raceme;

pedicels slender, 2.8 to 5.8 cm. long. Sepals 4, linear-lanceolate, 4 to 7 mm. long, unequal, acuminate, slightly constricted above base. Petals 4; claw up to 1 cm. long; blade narrowly oblong-elliptic, 2.3 to 5 mm. wide, up to 12 mm. long. Receptacle conspicuously elevated in center. Stamens 16 to 22; filaments up to 5 cm. long; anthers linear, 6 to 7 mm. long. Gynophore up to 5.5 cm. long. Fruits globose, up to 3.8 cm. in diam.

MEXICO—CAMPECHE: Palizada, swampside, July 25–28, 1939, *Eizi Matuda 3863* (TYPE in the University of Michigan Herbarium), a small tree, 7 m. high, 30 cm. in diam., flowers greenish-yellow, vernacular name *crusita*. TABASCO: Boca del Cerro on the Usumacinta River, swampside, July 1–5, 1939, *Matuda 3533,* a tree, 5 m. high, 25 cm. in diam. YUCATAN: km. 20, Merida-Progreso road, in thicket, July 27, 1938, *C. L. Lundell & Amelia A. Lundell 8142,* a tree, about 5 m. high, 15 cm. in diam., vernacular name *kolokmax;* Calotmul, *G. F. Gaumer 1679.* QUINTANA ROO: Coba, in forest covering the ruins, June 26, 1938, *Lundell and Lundell 7632,* a tree, about 6.5 m. high, 10 cm. in diam., fruits globose. BRITISH HONDURAS: El Cayo District, Little Cocquericot, Belize River, April 16, 1933, *C. L. Lundell 4189.*

C. glauca is nearest *C. gynandra* L. The small acuminate leaflets conspicuously glaucous on undersurface, the shorter petiolules, the larger sepals and petals, and fruits about 3.8 cm. in diameter distinguish it from that species. In the interpretation of *C. gynandra,* I have followed Fawcett and Rendle (Fl. Jam. **3**: 235. 1914).

C. Tapia L., which occurs in British Honduras (*Schipp 1150*), has non-glaucous leaflets and considerably larger flowers with the receptacle only slightly elevated.

Cassia (*Chamaecrista*) **itzana** Lundell, sp. nov. Frutex, ramulis gracilibus, pilosis. Stipulae ovatae, ad 8 mm. longae, 4 mm. latae, acuminatae. Foliola 5–13-juga, lineari-oblonga, 4–10 mm. longa, 1.2–3 mm. lata, supra glabra, subtus juventate pilosa, ciliata. Pedicelli ad 2.2 cm. longi. Sepala lanceolato-oblonga, apiculata, 6–8 mm. longa. Petala cuneato-obovata, ad 13 mm. longa. Legumen lineari-oblongum, ad 5.5 cm. longum, 4.2 mm. latum.

A low shrub, less than 1 m. high, branchlets slender, wiry, soft pilose, the hairs slightly incurved. Stipules conspicuous, prominently veined, ovate, up to 8 mm. long, 4 mm. wide, long acuminate, ciliate. Petiole bearing a small subsessile gland, the petiole and rachis together up to 3 cm. long, sparsely pilose. Leaflets chartaceous, 5 to 13 pairs, linear-oblong, 4 to 10 mm. long, 1.2 to 3 mm. wide, apex obliquely short aristulate, the costa submarginal, costa and veins conspicuous, glabrous above, at first pilose on under surface, persistently ciliate. Pedicels filiform, long pilose, up to 2.2 cm. long; bractlets subapical, lanceolate. Sepals lanceolate-oblong, 6 to 8 mm. long, inconspicuously veined, pilose. Petals cuneate-obovate, the largest 13 mm. long. Legume linear-oblong, up to 5.5 cm. long, 4.2 mm. wide, sparsely pilose.

MEXICO—YUCATAN: Progreso, east of port, in scrub on low sand dunes, July 23, 1938, *C. L. Lundell & Amelia A. Lundell 8055* (TYPE in the University of Michigan Herbarium), a low shrub, corolla yellow; same locality, west of port, in scrub on low sand dunes, July 17, 1938, *Lundell & Lundell 7959,* prostrate perennial, corolla yellow; kilometer 29, Merida-Progreso

road, in cleared marshy flats, July 26, 1938, *Lundell & Lundell 8127*, a shrub, about 60 cm. high, corolla yellow; Progreso, sandy plain behind beach, Aug. 11–15, 1932, *W. C. Steere 3100*.

C. itzana, referable to the *tristiculae* of the genus *Chamaecrista* according to Britton and Rose (N. Am. Fl. **23**: 270. 1930), is clearly allied to *C. jalapensis* (Britton) Lundell. The two sharply diverge in size of flowers and in number and size of leaflets.

Cassia jalapensis (Britton) Lundell, comb. nov. *Chamaecrista jalapensis* Britton, N. Am. Fl. **23**: 274. 1930.

Cassia yucatanensis (Britton & Rose) Lundell, comb. nov. *Peiranisia yucatensis* Britton & Rose, N. Am. Fl. **23**: 263. 1930.

Centrosema unifoliatum (Rose) Lundell, comb. nov. *Bradburya unifoliata* Rose. Contr. U. S. Nat. Herb. **8**: 45. 1903.

MEXICO—QUINTANA ROO: Coba, in savanna, July 1, 1938, *C. L. Lundell & Amelia A. Lundell 7746*, a perennial herbaceous vine, corolla lavender. TABASCO: Estapilla, near Tenosique, in savanna, June 27, 1939, *Eizi Matuda 3498*, a vine, corolla blue.

Mr. C. V. Morton, who has examined a specimen of the Matuda collection, refers the Tabasco material to this species. The banner in both the collections is more than twice as large as described by Rose.

Lonchocarpus Xuul Lundell, sp. nov. Frutex vel arbor parva, ramulis juventate parce sericeis. Folia 5–9-foliolata; foliola chartacea, glabra, oblonga, elliptica, vel ovata, 4–6 cm. longa, raro 3–11 cm. longa, 1.3–4.5 cm. lata, apice late obtusa, subacuminata, retusa, basi rotundata, petiolulis 3–4 mm. longis. Inflorescentiae racemiformes pauciflorae, rachi 2–6 cm. longa, parce sericea. Calyx late cupulatus, subinteger, 3 mm. altus, 5–6 mm. diam. Corolla parce sericea, vexillum suborbiculare, apice bilobatum, basi late cuneatum, ca. 10.2 mm. longum, 12 mm. latum. Ovarium lineare sericeum, ovulis 4 vel 5. Legumen 1–4-spermum.

A shrub, or tree up to 10 m. high, 20 cm. in diam., branchlets rather slender, at first sparsely short sericeous. Leaves 5- to 9-foliolate; the rachis slender, canaliculate, at first sparsely sericeous-puberulent, up to 9 cm. long. Leaflets chartaceous, pallid; the petiolules 3 to 4 mm. long, sparsely substrigillose at first; the blades oblong, elliptic, or ovate, usually 4 to 6 cm. long, sometimes 3 to 11 cm. long, 1.3 to 4.5 cm. wide, apex broadly obtuse, usually subacuminate, the acumen retuse, base rounded or sometimes acutish, usually slightly oblique, epunctate, sparsely strigillose above along costa, sparsely strigillose beneath, entirely glabrous with age, costa impressed above, prominent beneath, primary veins 7 to 9 on each side, prominulous beneath, inconspicuous above. Racemes appearing with the leaves, solitary at the nodes of young branchlets; the rachis rather sparsely sericeous with silvery hairs, 2 to 6 cm. long in flower, rarely up to 13.5 cm. long in fruit; peduncles and pedicels sparsely short sericeous, the peduncles solitary, biflorus, 2 to 2.3 mm. long, the pedicels about 1.5 mm. long. Bractlets oblong-elliptic, 1.3 mm. long. Calyx broadly cupulate, 3 mm. long, 5 to 6 mm. in diam., sparsely short sericeous. Standard petal dark green outside with purple margin, purplish within, rather sparsely short sericeous, suborbicular,

about 10.2 mm. long, 12 mm. wide, conspicuously bilobed at apex, base broadly cuneate, without basal lobes but appendaged within, the claw broad, short, only about 1.3 mm. long. Wings purplish, adhering to carina, oblique, rounded-auriculate, rounded at apex, claw 3 mm. long, blade 8 mm. long, 4 mm. wide. Carinal petals purplish, apex rounded and ciliolate, base acutish, not auriculate, the claw 3.5 mm. long, the blade 7 mm. long, 3.2 mm. wide, apical portion of wings and carinal petals sparsely sericeous, glabrous otherwise. Staminal tube glabrous, the vexillar stamen free at base. Ovary linear, cano-sericeous, 4- or 5-ovulate; style geniculate. Legume 1- to 4-seeded, slender stipitate, glabrous at maturity, up to 8 cm. long, 1.8 cm. wide, usually constricted between the seeds, the carinal suture flat, slightly broadened at the seeds, the vexillar suture thick, concave, about 6.5 mm. wide, narrowly winged on both sides (similar to legume of *L. sericeus* (Poir.) H.B.K.).

MEXICO—YUCATAN: Pisté-Yokdzonoot road, in advanced deciduous forest, July 11, 1938, *C. L. Lundell & Amelia A. Lundell 7864* (TYPE in the University of Michigan Herbarium), a tree, about 7 m. high, 7.5 cm. in diam., vernacular name *kanxuul;* near Xocenpich, in second growth, June 4, 1938, *Lundell & Lundell 7353,* a shrub, about 2 m. high; same locality and date, *Lundell & Lundell 7356*, a shrub, 1 m. high, standard petal dark green outside with purple margin, purplish within, wings and carinal petals purplish, vernacular name *xuul;* Chichen Itza, off Kaua road, in advanced deciduous forest, June 9, 1938, *Lundell & Lundell 7475*, a tree, about 10 m. high, 25 cm. in diam., corolla purple and green; same locality, June 9, 1938, *Lundell & Lundell 7481*, a tree, 8 m. high, 10 cm. in diam.; same locality and date, *Lundell & Lundell 7482*, a treelet, 4 m. high. BRITISH HONDURAS: Corozal District, in high ridge, 1931–1932, *Percy H. Gentle 417*, a tree, 7.5 cm. in diam., vernacular name *turtle-bone;* Corozal-Pachacan road, in high ridge, Aug. 11, 1933, *C. L. Lundell 4832,* a tree, 10 cm. in diam., vernacular name *turtle-bone.*

L. Xuul is referable to the subgenus *Neuroscapha*, series *Sericiflori* as delimited by Pittier (Contr. U. S. Nat. Herb. **20**: 37–93. 1917). It appears to be nearest *L. constrictus* Pittier.

The Motul Dictionary lists a *xul* tree which may be this species.

Trichilia yucatanensis Lundell, sp. nov. Arbor, ramulis substrigillosis et minute puberulis. Folia petiolata, minute puberula; foliola 3–7, raro 8, lanceolato-oblonga vel elliptico-oblonga, 3–15 cm. longa, 1.3–6.3 cm. lata, apice subabrupte acuminata, basi acuta, petiolulis ca. 4 mm. longis, raro 1 cm. longis. Inflorescentiae axillares, usque ad 8 cm. longae, substrigillosae et minute puberulae. Pedicelli 1.5–3 mm. longi. Calyx strigillosus. Corolla minute strigillosa, ca. 4 mm. longa, lobis triangulari-ovatis. Filamenta in tubum cylindricum 2.5 mm. longum connata, pilosa. Ovarium strigosum. Capsula obovoideo-ellipsoidea, ad 2 cm. longa, strigillosa.

A tree, up to 20 m. high, 35 cm. in diam., branchlet, petiole, and inflorescence substrigillose and minutely puberulent. Leaves usually odd-pinnate, the rachis densely but very minutely puberulent, the rachis and petiole 3 to 9 cm. long. Petiolules usually about 4 mm. long, rarely 1 cm. long. Leaflets alternate or subopposite, usually 3 to 7, rarely 8, lanceolate-oblong or elliptic-oblong, 3 to 15 cm. long, 1.3 to 6.3 cm. wide, apex subabruptly acuminate, the acumen usually obtusish, base acute, often inaequilateral, margin revolute, minutely and sparsely puberulent on upper surface, at first very sparsely

strigillose along costa on under surface and obscurely puberulent, primary veins 13 to 15 on each side, slender, prominulous beneath. Inflorescence axillary, paniculate, up to 8 cm. long, rather laxly flowered. Pedicels 1.5 to 3 mm. long, jointed at or below the middle. Calyx strigillose, about 1 mm. high, the lobes triangular, acutish. Corolla minutely strigillose, about 4 mm. long, united to the middle, the lobes triangular-ovate, acutish. Staminal tube 2.5 mm. long, pilose within, with a few hairs on the outside; anthers sessile, inserted at the bases of the sinuses, the lobes between the anthers filiform above middle. Ovary strigose. Capsule obovoid-ellipsoid, up to 2 cm. long, densely strigillose.

MEXICO—CAMPECHE: Tuxpeña, Dec. 10, 1931, *C. L. Lundell 1041* (TYPE in the University of Michigan Herbarium), a tree, 8 m. high; Monterrey, near Tuxpeña, Jan. 23, 1932, *Lundell 1240,* a tree, 20 m. high, vernacular name *mordal.*

The species, long confused with *T. moschata* Sw. of Jamaica, is abundant in the sapodilla forest of Campeche, northern Petén, eastern Tabasco, and British Honduras. From *T. moschata,* to which it has closest affinity, *T. yucatanensis* may be distinguished by its pilose staminal tube, flowers almost twice as large, longer pedicels, and minute heterotrichous indument. The leaf rachis and petiolule are consistently puberulent in all the specimens from the peninsula.

Symplococarpon flavifolium Lundell, sp. nov. Arbor parva, ramulis parce sericeis. Folia petiolata, flavida, chartacea, glabra, oblonga vel anguste elliptica, 4–7.5 cm. long, 1.5–2.5 cm. lata, apice obtusa, basi cuneata, margine serrulata. Flores ut videtur 1–3 in fasciculis axillaribus; pedicelli fructiferi glabri, recurvi, ad 11 mm. longi, bracteolis 2. Fructus ellipsoideus, subligneus, ca. 1.5 cm. longus, 1.1 cm. diam., calyce stylisque persistentibus coronatus, apice excepto glaber, bilocularis, 1–3-spermus.

A tree, 5 m. high, 15 to 20 cm. in diam.; branchlets slender, short, at first sericeous, drying blackish. Youngest leaves drying black, the petioles and undersurface of blades sparsely sericeous, mature leaves glabrous. Petioles canaliculate, 2 to 6.5 mm. long. Leaf blades chartaceous, yellowish, lucid above, oblong or narrowly elliptic, 4 to 7.5 cm. long, 1.5 to 2.5 cm. wide, apex usually obtuse, rarely obtusely subacuminate, the tip minutely notched, base cuneate, costa slightly impressed above, prominent beneath, primary veins 6 to 9 on each side, slender but prominulous on both surfaces, veinlets reticulate, most conspicuous above, margin serrulate. Flowers fasciculate, axillary, the fascicles apparently 1- to 3-flowered. Fruiting pedicels slender, recurved, up to 11 mm. long, the apical bracteoles alternate or subopposite, sparsely appressed hairy. Fruits indehiscent, ellipsoid, about 1.5 cm. long, 1.1 cm. diam., crowned by the 5 persistent calyx lobes; apical superior part hirsute; 2-celled, 1- to 3-seeded.

MEXICO—CHIAPAS: Las Cadenas near Escuintla, Jan., 1938, *Eizi Matuda 1883* (TYPE in the University of Michigan Herbarium), a tree, 5 m. high, 15 to 20 cm. in diam.; Mt. Ovando, April 5, 1936, *Matuda 696.*

Both of the collections are in fruit, and disposition of the material has been a puzzle to the writer for over three years. *S. flavifolium* appears to be nearest *S. multiflorum* Kobuski. The oblong obtuse yellowish chartaceous leaf blades conspicuously reticulate veined, the sericeous indument of the

young parts, and the hirsute superior portion of the ovary are distinguishing characteristics.

Symplococarpon lucidum Lundell, sp. nov. Arbor parva, glabra. Folia petiolata, membranacea vel chartacea, supra lucida, elliptico-oblonga, oblanceolata, vel lanceolata, 4–10 cm. long, 2–4.3 cm. lata, apice obtuse acuminata, basi cuneata, margine crenato-serrata. Flores ad 9 in fasciculis axillaribus; pedicelli glabri, recurvi, bracteolis 2, oppositis vel suboppositis. Hypanthium glabrum, calycis lobis 5, ciliolatis. Petala 5. Stamina 28 vel 30. Ovarium fere totum inferius, biloculare, ovarii parte superiore hirsuta; styli 2, liberi.

A tree, 17.5 cm. in diam., buds white strigillose, entirely glabrous otherwise; branchlets slender, wiry, reddish. Petioles shallowly canaliculate, rather stout, 2.5 to 4.5 mm. long. Leaf blades membranaceous or chartaceous, usually shining above, yellow-green, elliptic-oblong, oblanceolate, or lanceolate, 4 to 10 cm. long, 2 to 4.3 cm. wide, apex obtusely acuminate, base cuneate, costa plane above, prominent beneath, primary veins 6 to 9 on each side, prominulous, veinlets reticulate, the reticulation prominulous beneath, less conspicuous above, margin rather remotely crenate-serrate. Flowers fasciculate, axillary, the fascicles up to 9-flowered; pedicels (of buds) slender, up to 1 cm. long, usually recurved, bearing two opposite or subopposite bracteoles at apex, the bracteoles ovate, 0.6 to 0.8 mm. long, ciliolate. Hypanthium glabrous. Calyx lobes 5, imbricate, depressed-orbicular, ciliate. Petals 5. Stamens 28 or 30; anthers tapering at apex into a subulate mucro. Superior portion of ovary sparingly hirsute. Styles 2, free. Ovary inferior, 2-celled, with 2 or 3 ovules in each cell.

British Honduras: Stann Creek District, Stann Creek Valley, Mountain Cow Ridge, in high ridge, Mar. 10, 1940, *Percy H. Gentle 3248* (type in the University of Michigan Herbarium), bark dark green, wood pink, vernacular names *pasa macho, wild raisin male.*

Although the material is in bud only, it obviously represents an undescribed species closely allied to *S. multiflorum* Kobuski. The thin leaf blades with rather remote crenatures distinguish *S. lucidum.*

Kobuski in his recent treatment of the genus (Journ. Arn. Arb. **22**: 188. 1941) states in the generic description "ovules solitary in each cell." The ovary of *S. lucidum* has 2 or 3 ovules in each cell, and in *S. multiflorum* (*Stork 2305*) no less than 7 ovules are present in some cells.

Hypericum Matudai Lundell, sp. nov. Frutex glaber. Folia petiolata, chartacea, pellucido-punctata, oblonga vel lanceolato-oblonga, 1–3.2 cm. longa, 0.6–1.4 cm. lata, apice rotundata, basi acutiuscula, biauriculata. Inflorescentiae cymosae. Pedicelli 3–5 mm. longi. Sepala 5, striata, ovato-elliptica, 2.5–3 mm. longa. Petala oblonga, ad 7 mm. longa, 2.6 mm. lata. Stamina 9, fasciculata. Ovarium 3-loculare. Capsula ca. 4 mm. longa.

A glabrous shrub, branchlets slender, short, crowded, with very short internodes. Petioles canaliculate, up to 3.5 mm. long. Leaf blades chartaceous, pellucid-punctate, oblong or lanceolate-oblong, 1 to 3.2 cm. long, 0.6 to 1.4 cm. wide, apex rounded, base acutish and decurrent, biauriculate, the auricles incurved, essentially entire, primary veins usually 4 on each side, inconspicuous. Inflorescence cymose, the cymes up to 3.5 cm. long; bractlets lanceolate, up to 2.5 mm. long, acuminate, minutely denticulate. Pedicels

slender, thickened above, 3 to 5 mm. long. Sepals 5, striate, ovate-elliptic, 2.5 to 3 mm. long, minutely denticulate-ciliolate, rounded at apex. Petals oblong, up to 7 mm. long, 2.6 mm. wide. Stamens 9, in fascicles of three, the fascicles alternating with glands; filaments up to 4 mm. long. Styles 3, slender. Ovary 3-celled. Capsule about 4 mm. long.

MEXICO—CHIAPAS: Mt. Paxtal, Dec. 29, 1936, *Eizi Matuda 499* (TYPE in the University of Michigan Herbarium).

AMMANNIA KOEHNEI Britton, Bull. Torrey Bot. Club **18**: 271. 1891.

MEXICO—YUCATAN: east of Sisal, growing with *Acrostichum* in mangrove forest on island in the *cienaga*, July 29, 1938, *C. L. Lundell & Amelia A. Lundell 8188*, an erect herb.

The species apparently has not been found heretofore in Mexico and Central America. It ranges from New Jersey to Florida, and the extension into Yucatan is not surprising. The plant closely resembles *A. latifolia* L., an apetalous species. Only two flowers are present on the Yucatan material, but both bear four small fugacious petals.

ROTALA RAMOSIOR (L.) Koehne var. **dentifera** (A. Gray) Lundell, comb. nov. *Ammannia dentifera* A. Gray, Pl. Wright. in Smithson. Contr. **5**: 55. 1853.

BRITISH HONDURAS: Toledo District (?), Forest Home, alt. about 50 m., in open swampy places, Dec. 18, 1933, *W. A. Schipp S-492*, a weedy annual, flowers pink.

The longer appendices of the calyx appear to be the only important characteristic for the segregation of *R. dentifera* (A. Gray) Koehne as a distinct species. This characteristic can scarcely be considered of more than varietal importance.

Eugenia itzana Lundell, sp. nov. Arbor parva, ramulis glabris. Folia glabra, petiolata, chartacea, lanceolata, ovato-lanceolata, vel elliptico-oblonga, 3–6.5 cm. longa, 1.1–3.8 cm. lata, apice obtusa, basi acutiuscula, costa supra immersa. Inflorescentiae axillares, racemosae, densiflorae, rachidibus ad 6 mm. longis. Pedicelli 1–3 mm. longi. Petala elliptica, 3 mm. longa, ciliolata.

A small tree, up to 4 m. high, 5 cm. in diam.; branchlets slender, glabrous, compressed at the nodes, the internodes terete; branches whitish. Petioles slender, 3 to 7 mm. long, glabrous. Leaf blades chartaceous, glabrous, lanceolate, ovate-lanceolate, or elliptic-oblong, 3 to 6.5 cm. long, 1.1 to 3.8 cm. wide, apex obtuse, base acutish, costa impressed above, prominent beneath, primary veins 5 to 7 on each side, evident but scarcely prominulous. Inflorescence axillary, short racemose, the rachis up to 6 mm. long, usually less than 5 mm. long, sparsely puberulent at first. Pedicels slender, 1 to 3 mm. long, sparsely puberulent at first, usually glabrous very early. Calyx minute, the tube about 1 mm. long, glabrous; lobes 4, broadly ovate, 0.5 to 0.8 mm. long, ciliolate. Petals elliptic, 3 mm. long, ciliolate.

MEXICO—YUCATAN: Chichen Itza, off Kaua road, in advanced deciduous forest, June 16, 1938, *C. L. Lundell & Amelia A. Lundell 7589* (TYPE in the University of Michigan Herbarium), a treelet, 3 m. high, 2.5 cm. diam., petals white; near Pisté, in forest, June 18, 1932, *W. C. Steere 1373*, ver-

nacular name *chak-ni*. QUINTANA ROO: Coba, west end of Lake Coba, in second growth, June 28, 1938, *Lundell & Lundell 7687,* a treelet, 4 m. high, 5 cm. in diam.; east of Coba ruins, in advanced deciduous forest, July 5, 1938, *Lundell & Lundell 7818,* a small tree.

E. itzana may be separated from *E. balancanensis* Lundell, the closest species, by its broader leaves with costa immersed above, pedicels shorter and usually puberulent at first, and calyx with lobes only 0.5 to 0.8 mm. long. The calyx lobes of *E. balancanensis* are fully twice as large .

Eugenia leptopa Lundell, sp. nov. Arbor parva, ramulis glabris. Folia glabra, petiolata, coriacea, lanceolata, lanceolato-oblonga, anguste elliptica, vel ovato-elliptica, 2.5–6 cm. longa, 0.8–3.1 cm. lata, apice attenuata, obtusa, basi acuta. Flores axillares, fasciculati, pedicellis fructiferis 5–16 mm. longis, glabris. Fructus subglobosus, ca. 1 cm. diam.

A small slender tree, up to 4 m. high, 4 cm. in diam.; branchlets slender, glabrous, compressed at nodes; branches terete, whitish. Petioles slender, canaliculate, 3.5 to 6 mm. long, glabrous. Leaf blades rigidly coriaceous, paler beneath, glabrous, lanceolate, lanceolate-oblong, narrowly elliptic, or ovate-elliptic, 2.5 to 6 cm. long, 0.8 to 3.1 cm. wide, apex attenuate and obtuse, base acute, costa slightly impressed above, prominent beneath, primary veins 5 or 6 on each side, scarcely prominulous on under surface, obscure above, conspicuously punctate. Flowers fasciculate in the leaf axils; pedicels slender, in fruit 5 to 16 mm. long, glabrous. Fruits subglobose, about 1 cm. in diam., obscurely costate, glabrous. Persistent calyx lobes 4, unequal, suborbicular, 2.5 to 3.3 mm. long, broadly rounded, the margin ferruginous-puberulent.

MEXICO—YUCATAN: off Pisté-Yokdzonoot road, in advanced deciduous forest, July 11, 1938, *C. L. Lundell & Amelia A. Lundell 7869* (TYPE in the University of Michigan Herbarium), a treelet, 4 m. high, bark white and smooth, fruits subglobose, dark red; near Telchac, in scrub on low sand dunes, July 25, 1938, *Lundell & Lundell 8111,* a treelet, about 2 m. high; near Yokdzonoot, in advanced deciduous forest, July 14, 1938, *Lundell & Lundell 7927,* a treelet, 3 m. high, 4 cm. in diam., vernacular name *saclob*.

The leaves of numbers *7927* and *8111* are somewhat wider and the pedicels shorter than in the type. The affinity of *E. leptopa* is with *E. Lundellii* Standl.

Eugenia ovatifolia Lundell, sp. nov. Arbor parva, ramulis subadpresse rufo-puberulis. Folia petiolata, subcoriacea, pallida, ovata vel ovato-elliptica, 6–9.8 cm. longa, 3–5.8 cm. lata, apice subabrupte subacuminata, acumine obtuso, basi late rotundata. Inflorescentiae axillares, racemosae, densiflorae, rachidibus ad 5 mm. longis. Pedicelli parce puberuli, ad 3.5 mm. longi. Petala late elliptica, ca. 3.2 mm. longa, parce ciliolata. Fructus subglobosus, verrucosus.

A small tree, about 4 m. high, 7.5 cm. diam.; branchlets rather stout, compressed at the nodes, at first subsericeous with short subappressed rufous hairs; branches glabrous, terete, becoming whitish. Petioles stout, canaliculate, 3 to 6 mm. long, subsericeous. Leaf blades subcoriaceous, pallid, at first sparsely sericeous along the costa on both surfaces, glabrous early, ovate or ovate-elliptic, 6 to 9.8 cm. long, 3 to 5.8 cm. wide, apex subabruptly short

acuminate, the acumen broadly obtuse, base rounded and slightly decurrent, costa impressed above, prominent beneath, primary veins 7 or 8 on each side, prominulous on both surfaces, veinlets laxly reticulate. Inflorescence axillary, short racemose, the racemes fasciculate, crowded, the rachis up to 5 mm. long, usually only 2 to 3 mm. long, bractlets rufous-pubescent. Pedicels sparsely puberulent, up to 3.5 mm. long. Calyx tube glabrous, scarcely 1 mm. long; lobes 4, minute, unequal, broadly rounded, 0.4–0.8 mm. long, rufous-ciliolate. Petals broadly elliptic, about 3.2 mm. long, sparsely ciliolate. Stamens up to 4 mm. long; anthers with red spot at apex. Fruits subglobose, verrucose when young.

BRITISH HONDURAS: Belize District, Belize-Sibun River road, 1931–32, *Percy H. Gentle 30* (TYPE in the University of Michigan Herbarium), a small tree, about 4 m. high, 7.5 cm. diam., flowers white; on bank of the Sibun River, Nov. 15, 1934, *Gentle 1401*, a tree, 7.5 cm. diam., vernacular name *blossom berries*.

Eugenia petenensis Lundell, sp. nov. Frutex vel arbor parva, ramulis puberulis. Folia petiolata, petiolo puberulo, lamina chartacea, ovata, ovato-elliptica, vel lanceolata 3.5–8.5 cm. longa, 2.1–4.3 cm. lata, apice acuminata vel subacuminata, acumine obtuso vel obtusiusculo, basi acuta. Flores axillares, solitarii vel in racemi dispositi. Pedicelli parce puberuli vel glabri, 6–15 mm. longi. Petala ovato-elliptica, 4 mm. longa, ciliata. Fructus globosus, ca. 7 mm. diam.

An arborescent shrub or small tree; branchlets slender, subcompressed at nodes, terete early, puberulent; branches whitish. Petioles canaliculate, puberulent, 1.5 to 4 mm. long. Leaf blades chartaceous, concolorous, ovate, ovate-elliptic, or lanceolate, 3.5 to 8.5 cm. long, 2.1 to 4.3 cm. wide, apex acuminate or short acuminate, the acumen usually obtuse or obtusish, base acute, costa and primary veins impressed above, the costa prominent beneath, the primary veins prominulous, 6 to 9 on each side, sparsely puberulent along the costa, glabrous otherwise. Flowers solitary, short racemose, or in leafy few-flowered elongated racemes up to 4.5 cm. long, axillary, the rachis sparsely puberulent. Pedicels sparsely puberulent or glabrous, very slender, 6 to 15 mm. long. Calyx glabrous; tube scarcely 1 mm. long; lobes 4, unequal, the larger ovate-elliptic, the smaller depressed ovate, 1 to 2 mm. long, ciliate. Petals ovate-elliptic, 4 mm. long, ciliate. Fruits globose, about 7 mm. in diam.

GUATEMALA—PETÉN: La Libertad, in marginal forest, June 10, 1933, *C. L. Lundell 3746* (TYPE in the University of Michigan Herbarium), a shrub, vernacular name *jolteillo;* same locality, June 5, 1933, *Lundell 3631*, a small tree; same locality, bordering an *aguada*, April 5, 1933, *Lundell 2504*, a small tree. BRITISH HONDURAS: El Cayo District, Cocquericot, Mar. 19, 1931, *H. H. Bartlett 12063, 12064*.

Yuncker, Dawson, and Youse 5765 from Honduras appears to be referable here also. *E. petenensis* is nearest *E. cocquericotensis* Lundell, but differs amply from that species in its solitary or openly racemose long pedicelled flowers. In *E. cocquericotensis* the racemes are congested and the pedicels do not exceed 3 mm. in length. The two species well illustrate the transition from solitary axillary flowers to a racemose inflorescence.

On the basis of determinations by Mr. P. C. Standley, *Lundell 3746* and

3631 have been reported as *E. vincentina* Krug & Urban, and *Lundell 2504* as *E. xalapensis* (H.B.K.) DC. (Carnegie Inst. Wash., Publ. 478: 179. 1937).

Stylogyne perpunctata Lundell, sp. nov. Frutex vel arbor parva, glabra. Folia longe petiolata, petiolo marginato, ad 3 cm. longo, lamina integra, membranacea, pellucido-punctata, elliptica vel obovato-elliptica, 10–15 cm. longa, 5–7 cm. lata, apice abrupte subacuminata, basi cuneata. Inflorescentiae terminales, sessiles, ad 6 cm. longae. Pedicelli ad 7 mm. longi. Sepala 5, lanceolato-oblonga, ca. 2 mm. longa, minute erosa. Corolla 5 mm. longa; petala 5, oblonga, apice oblique emarginata. Fructus 6 mm. diam.

A shrub or small tree, buds minutely scaly, entirely glabrous otherwise. branchlets terete, slender. Petioles marginate, slender, 1.2 to 3 cm. long. Leaf blades entire, membranaceous, conspicuously pellucid-punctate, elliptic or obovate-elliptic, 10 to 15 cm. long, 5 to 7 cm. wide, apex abruptly short acuminate, the acumen triangular, less than 1 cm. long, base cuneate and decurrent, costa plane above, prominent beneath, reticulate veined, the primary veins slender, rather conspicuous on under surface, less evident above. Inflorescence terminal, bipinnate, sessile, reddish, up to 6 cm. long, the flowers umbellate, white, fragrant. Pedicels slender, usually curved, 3 to 7 mm. long. Sepals 5, united at base, lanceolate-oblong, about 2 mm. long, scarious, minutely erose, often obliquely emarginate at apex. Corolla 5 mm. long; petals 5, oblong, united at base into a tube 1.5 mm. long, reddish-punctate, hyaline, obliquely emarginate at apex. Filaments about 1 mm. long, attached near base of tube, the basal third blackish, apparently glandular. Anthers 2 mm. long. Berries globose, 6 mm. in diam.

BRITISH HONDURAS: Stann Creek District, Silk Grass Creek Reserve, in second growth (*acahual*), Sept. 10, 1939, *Percy H. Gentle 2990* (TYPE in the University of Michigan Herbarium), a small tree, vernacular name *pigeon berries;* Stann Creek Valley, Big Eddy Ridge, in broken ridge, April 8, 1941, *Gentle 3552,* a shrub, flowers white, fragrant.

According to description, *S. guatemalensis* Blake is the closest ally. The membranaceous leaf blades, the marginate petioles up to 3 cm. long, the pedicels usually 4 to 7 mm. long, the minutely erose scarious sepals, and the hyaline petals obliquely emarginate distinguish *S. perpunctata*. The leaves are very conspicuously pellucid-punctate. *S. guatemalensis* is described as having coriaceous leaves with unmargined petioles 5 to 12 mm. long, pedicels only 2 to 3.5 mm. long, entire sepals, and petals obtuse or rounded at apex.

The vernacular name *pigeon berries* is applied in British Honduras to various plants with small edible berries.

Matelea stenosepala Lundell, sp. nov. Scandens, caulibus ut petiolis pedunculisque parce hirsutis, dense puberulis, et minute rufo-tuberculato-puberulis. Folia petiolata, membranacea, ovato-cordata, 5–10.5 cm. longa, 4.5–7.8 cm. lata, apice abrupte caudato-acuminata, basi cordata, supra puberula, subtus puberula et parce hirsuta. Pedunculi axillares, 2–3 cm. longi. Flores subumbellati, pedicellis ad 3 cm. longis. Sepala lineari-lanceolata, ad 6.5 mm. longa. Corolla atro-rubra, puberula.

An herbaceous vine, about 2 m. high; stems, petioles, peduncles, and pedicels sparsely hirsute, densely puberulent, and covered with minute red

bulbous hairs. Petioles slender, up to 6 cm. long. Leaf blades membranaceous, slightly paler beneath, ovate-cordate, 5 to 10.5 cm. long, 4.2 to 7.8 cm. wide, apex abruptly caudate-acuminate, the acumen narrow, up to 8 mm. long, base deeply cordate, primary veins 5 or 6 on each side, slender, densely puberulent beneath with intermixed bulbous hairs, sparsely hirsute and puberulent above, the minute red bulbous hairs scattered. Peduncle slender, 2 to 3 cm. long, few-flowered. Bractlets linear, up to 6 mm. long. Pedicels filiform, up to 3 cm. long. Sepals linear-lanceolate, up to 6.5 mm. long, with outside indument like that of pedicels, glabrous within. Corolla dark red, almost black, the lobes ovate-lanceolate, 6.5 mm. long, puberulent on both surfaces, densely so within. Follicles smooth, with indument like that of stems.

MEXICO—QUINTANA ROO: Coba, in low second growth, June 27, 1938, *C. L. Lundell & Amelia A. Lundell 7648* (TYPE in the University of Michigan Herbarium).

Tournefortia belizensis Lundell, sp. nov. Frutex, ramulis hirtellis. Folia petiolata, membranacea vel subchartacea, hirtella, anguste cuneato-oblanceolata, 9.5–17 cm. longa, 3–4.7 cm. lata, apice acuta vel acuminata, basi attenuata, acuminata. Inflorescentiae hirtellae, pedunculatae, cymosae; flores spicati. Calyx 3 mm. longus. Corolla puberula, ca. 6 mm. longa, tubo cylindrico ca. 4 mm. longo, lobis lanceolatis, acutis.

Erect shrub, 2 m. high, simple or with few branches, branchlets densely hirtellous, inconspicuously angled at first. Petioles canaliculate, up to 6 mm. long, hirtellous. Leaf blades membranaceous or subchartaceous, persistently hirtellous on both surfaces, narrowly cuneate-oblanceolate, 9.5 to 17 cm. long, 3 to 4.7 cm. wide, apex acute or acuminate, base narrowly attenuate, decurrent, paler beneath, primary veins 6 or 7 on each side. Flowers in scorpoid cymose spikes; spikes 2 to 5 in the inflorescence, up to 10 cm. long, hirtellous. Calyx 3 mm. long, shorter than the corolla tube; sepals linear-lanceolate, attenuate to an acute apex, sparsely hirtellous. Corolla about 6 mm. long, puberulent outside; tube cylindrical, about 4 mm. long; lobes lanceolate, acute, scarcely 2 mm. long. Anthers sessile, inserted at middle of corolla tube. Ovary and style glabrous or sometimes bearing a few scattered short hairs.

BRITISH HONDURAS: El Cayo District, El Cayo, in forest on hillside along the Macal River, June 18, 1936, *C. L. Lundell 6151* (TYPE in the University of Michigan Herbarium).

T. belizensis is closely allied to *T. umbellata* H.B.K., a species with glabrous leaves.

UNIVERSITY OF MICHIGAN
ANN ARBOR, MICHIGAN

INDEX TO AMERICAN BOTANICAL LITERATURE

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Papers that relate exclusively to bacteriology, forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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BULLETIN OF THE TORREY BOTANICAL CLUB
69(6): 421–428 JUNE, 1942

THIAMINE IN SOME COMMON AMERICAN TREES[1]

P. R. BURKHOLDER AND A. G. SNOW, JR.

The importance of thiamine (vitamin B_1) in metabolism and growth of plants and animals has become well established in recent years. Comparatively little quantitative work has been done, however, with the vitamin relations of woody plants. Dagys (1936) demonstrated in expanding buds of trees the presence of a substance which stimulates cell division in cultures of yeast. Enders and Hegendorfer (1941) similarly reported on yeast growth factors contained in trees. Growth stimulation of *Phycomyces* by extracts from leaves of many species of trees was described by Schopfer (1936). The vitamin B_1 content of leaves of several woody species grown in sand or soil culture in California was studied by Bonner and Greene (1939) with a modification of Schopfer's method. These authors claimed that species such as *Camellia japonica* and *Daphne odora*, which are low in naturally occurring vitamin B_1, can be stimulated to grow more rapidly by supplying synthetic thiamine to their roots. Other investigators have not been able to obtain any growth-promoting effects by the addition of synthetic thiamine to the roots of many species (Hamner 1940). In view of the widespread biological importance of thiamine, further knowledge concerning the occurrence of this vitamin in trees may be of practical significance for certain problems in forest genetics, silviculture, and wild life management.

DISCUSSION OF METHODS

Of the known methods available for determination of thiamine in plant tissues, one very sensitive and simple quantitative test involves the use of the fungus *Phycomyces blakesleeanus*. Growth of this fungus is controlled largely by the amount of thiamine (Schopfer 1934) or its components, pyrimidine and thiazole, supplied in culture media (Robbins and Kavanagh 1937, 1938a, 1938b; Bonner and Erickson 1938; Bonner and Buchman 1939). The *Phycomyces* assay method measures the cumulative activity of thiamine, its two components, and cocarboxylase. All of these are active too in the metabolism and growth of higher plants, but animals appear to use only the whole thiamine molecule (Harris 1938). It is known also that unidentified substances in tissue extracts added to synthetic media may stimulate somewhat the growth of the fungus in the presence of thiamine (Robbins and Hamner 1940). Hence it should be recognized that this biological assay method gives only an approximation of the thiamine and related compounds

[1] Joint contribution from Osborn Botanical Laboratory, Yale University, and Northeastern Forest Experiment Station, New Haven, Conn.

contained in tissues. However, the reliability of the method has been tested by Bonner and Greene (1939) who reported that known amounts of thiamine added to ground leaf tissue could be recovered quantitatively in the *Phycomyces* test.

In our experiments it was found that thiamine added to preparations of plant material, such as elm buds, etc., could be recovered. The accuracy with which the method allowed recovery of synthetic thiamine from media containing extracts of tissues varied somewhat among the species of trees selected for study. The recovery from extracts of some species, such as sugar maple, was very inefficient. It appears that growth-inhibiting substances as well as growth-promoting factors must be recognized in evaluating results obtained with the *Phycomyces* method. Some of the difficulties encountered in using the method have been emphasized earlier by Sinclair (1938).

The methods used for determining the approximate thiamine activity of buds, leaves, and bark of trees at different seasons of the year are essentially those which were reported in an earlier paper (Burkholder and McVeigh 1940). For each analysis approximately 100 mg. of fresh tissue were weighed rapidly on a dampened analytical balance, and ground fine in the acid nutrient solution with a glass mortar and pestle. The nutrient solution containing the ground tissue was placed in 125 ml. Erlenmeyer flasks plugged with cotton, and autoclaved at 15 pounds for 15 minutes. Inoculation was made from a pure spore suspension of *Phycomyces*. After growing at 20° C. for 10 days dry weights of the washed fungus mats were determined. By reference to standard growth curves obtained with the fungus cultivated in a series of synthetic nutrient solutions with known amounts of thiamine, the approximate thiamine activity of the samples of tissue was estimated. Although it seems probable that most of our estimated data may be somewhat too high because of the growth-stimulatory factors other than thiamine presumably supplied by the tissues, it is thought that the assays possess comparative value. Replications during sampling made it possible to determine the statistical significance of the data according to the methods of Fisher (1936).

RESULTS

Inhibition of Growth of Phycomyces. Preliminary experiments with *Phycomyces blakesleeanus* indicated that growth of the fungus is inhibited by addition to the culture medium of materials from certain deciduous trees. Extracts from species such as *Quercus alba* and *Acer saccharum* produced particularly strong inhibition of growth. A comparative study was then made on the effects of ground leaves of sugar maple, Maryland Mammoth tobacco, and Marglobe tomato upon growth of *Phycomyces* in the basal medium containing 10^{-6} M synthetic thiamine. Three replications of each

treatment were made and after growth for 10 days the response of the fungus was determined by measuring the dry weight of the mats. The amount of fresh tissue added to each culture flask varied from 25 to 800 mg. Controls were run with no tissues added to the basal medium.

Some of the results obtained in these experiments are shown graphically in figure 1. Increasing the supply of ground tissues of these particular vari-

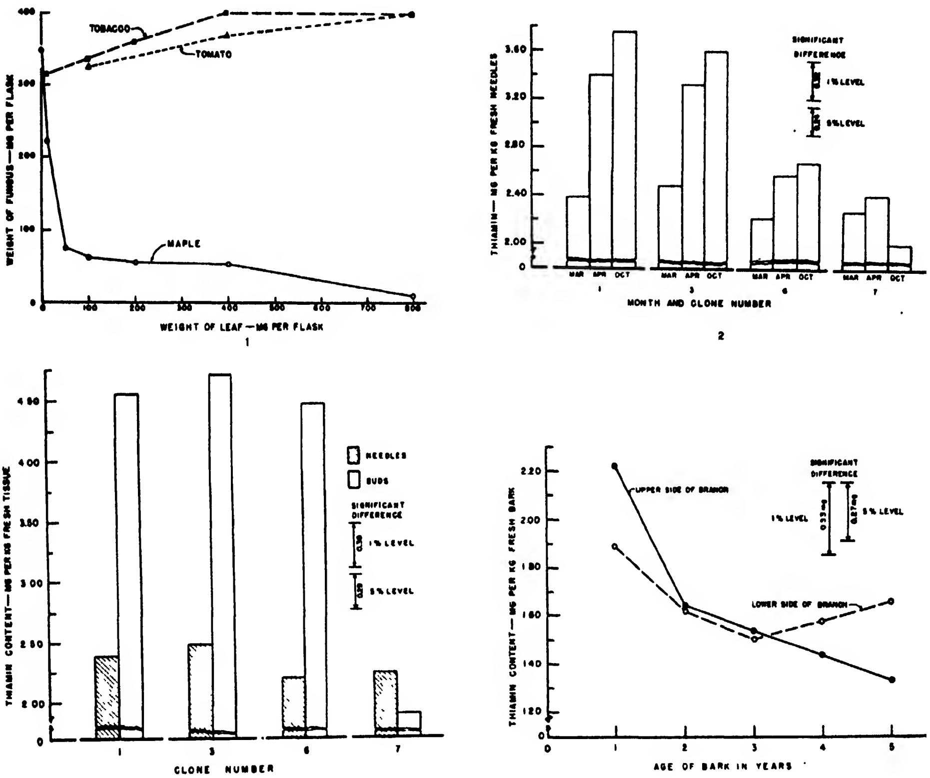

FIG. 1. Influence of increments of ground fresh leaf tissue of Maryland Mammoth tobacco, Marglobe tomato, and sugar maple on growth of *Phycomyces* in nutrient solution containing synthetic thiamine. Sugar maple inhibits growth of the fungus. FIG. 2. Thiamine activity in needles of four white pine trees sampled on March 15, April 30, and October 11, 1941. Significant differences occur among the individual trees and at different times. FIG. 3. Thiamine activity in needles and buds of white pine trees sampled on March 15, 1941. Significant differences occur between needles and buds of trees number 1, 3, and 6, but not in tree 7. FIG. 4. Distribution of thiamine in bark of white pine branches sampled along the upper and lower sides at 5 levels from tip to base on May 7, 1941.

eties of tobacco and tomato exerted no deleterious effect on growth of the fungus, but actually brought about some increase in dry weight. In contrast with cultures containing these tissues, the addition of even small amounts of

leafy material from sugar maple greatly reduced the dry weight of the fungus. It is obvious that the simplified *Phycomyces* assay for thiamine can not be employed for tissues of all kinds of plants. It seems probable that unidentified substances present in leaves of sugar maple may be responsible for inhibition of growth of *Phycomyces*. There is some evidence to indicate growth-inhibiting substances also in buds of Norway maple and in species of oak. No evidence of such inhibitory action was found in red maple or any of a number of other species of trees.

Stimulation of Growth of Phycomyces by Extracts from Evergreen and Deciduous Trees. Studies were made on the thiamine activity of needles, buds, and bark samples taken from a number of selected, 12-year-old white pine trees in North Branford, Connecticut. The purpose of the investigations was to obtain some indication of the occurrence of the vitamin in different parts of evergreen trees, the possible variation among different individual trees of the same species, and the extent of fluctuation in vitamin content at different times during the year. Approximately 100 mg. of fresh needles, bark, or buds were used for each assay.

Investigations of individual variation in white pine involved the sampling of 10 different trees which are being used as source material for establishing clones by vegetative propagation. The results of assays on the vitamin B_1 content of fresh needles of four of these trees are reported here to indicate the nature of the variations found in response of *Phycomyces* to this coniferous material. A more extensive report on growth habits and distribution of thiamine in these trees is planned for a later paper. Needles of the last season's growth were collected from three branches of 1937 origin on each tree, and after thorough mixing three random samples were taken for replicate assays. The calculated values for thiamine in the needles of trees number 1, 3, 6, and 7, sampled March 15, April 30, and October 11, 1941, are indicated in figure 2. Trees 1 and 3 were richer in thiamine than were 6 and 7. These four examples represent the rich and poor types in the group of 10 trees studied. It appears that in those trees which showed a higher content of thiamine in early spring the vitamin content increased considerably during the growing season. Trees 1 and 3 showed significant increases in vitamin from March through April to October, but trees 6 and 7 exhibited little or no significant increases with advance of the summer season. It must be admitted here that any correlation of thiamine content with growth and general vigor of the trees must await further study.

On October 11, 1941, two grams of fresh leaf material from each tree were oven-dried at 70° C. and weighed to allow the calculation of thiamine on a dry weight basis. Since it was found that the dry matter was in all the samples taken about 42 per cent of the fresh weight, it would seem to make

little difference whether the thiamine values are expressed on the basis of the fresh or dry weight of tissues from these trees.

Determinations of thiamine in the buds of the trees were made on March 15, 1941. A comparison of the data for needles and buds is presented in figure 3. In trees 1, 3, and 6, the vitamin was far more abundant in the buds than in the leaves, but in tree number 7 there was little significant difference between the values for the two regions.

On May 7, 1941, the distribution of thiamine in the 1-, 2-, 3-, 4-, and 5-year-old bark of three white pine trees was determined with 3 replicate samples from each region selected from both the upper and lower sides of a 5-year-old branch. The averaged data are shown graphically in figure 4. A gradient in vitamin distribution exists in the upper side of the branches ranging from a high concentration in the 1-year-old bark taken from near the tip of the branch downward to the 5-year-old portion of the branch. This significant trend in the upper side was not found in the lower side of these same branches. Just what meaning the data may have for growth and development is not clear. The facts do indicate the need for careful sampling technique in any attempt to analyze the vitamin distribution in trees.

The buds and leaves of eight species of deciduous trees located on the Yale campus were examined for thiamine activity in six different months during 1941. Three replicate determinations were made on each kind of fresh material assayed on each of six sampling dates.

The averaged data for estimated thiamine in buds and leaves of these trees are presented in table 1. The values expressed as milligrams of thiamine

TABLE 1

Estimated thiamine (as mg. per kg. fresh tissue) in buds and leaves of trees sampled at different times during 1941. The blanks indicate no assays

Date sampled (1941)	3/24	4/18	5/9	7/12	10/12	11/25
	Buds	Buds	Leaves	Leaves	Leaves	Buds
Aesculus hippocastanum	4.4	4.0	4.4	3.5	5.5	5.6
Fagus sylvatica	6.1	6.8	6.4	4.4	4.9	
Platanus occidentalis	4.1	3.7	3.7	...	2.3	...
Acer platanoides	0.0	1.4	2.3		2.8	...
Acer rubrum	...	4.6	2.8	3.3	3.5	7.4
Ulmus americana	...	3.4	3.3	2.9	3.1	6.3
Quercus palustris	3.3	0.6	0.0	...	0.0	3.7
Cornus florida (flowering branch)	0.6	0.2	0.2	...	...	...
Cornus florida (vegetative branch)	3.5	1.3	1.0	...	2.5	...

per kilogram of fresh tissues show some variation among the species. The assays for *Quercus palustris* tended to be low during the summer months.

A few determinations made on *Q. alba* produced very little growth of *Phycomyces*. As mentioned above, it seems probable that unidentified substances in the oaks and some maples interfere with the assay by inhibiting growth of the fungus. Leaves and buds from flowering branches of *Cornus florida* also permitted but little growth of the fungus as compared with the vegetative shoots from another tree of the same species. The data indicate in general a widespread occurrence of thiamine in the buds and leaves of trees at different seasons of the year. The buds of red maple and elm contained larger amounts of the vitamin in the fall than when tested in the spring. The values in general agree well with biological assays on certain woody plants as reported by Bonner and Greene (1939).

Distribution of Thiamine in Ringed Trees. Some preliminary experiments were performed to determine whether removing a ring of bark from trees would influence the distribution of thiamine. Young trees of basswood, red maple, and white pine were girdled by removing a ring of bark from the lower portion of the main trunk. The bark was assayed for thiamine at the time of ringing. After the lapse of from 1 to 4 months samples of the bark just above and below the ring were assayed.

Five 3-year-old white pine trees were ringed on March 15 and tested again on April 30, 1941. After the girdling operation depletion of the vitamin was greater above than below the ring at this season of the year. It is suggested that perhaps some form of thiamine or its precursors stored in the plant body aided in maintaining a comparatively high value for a while in the bark. Unfortunately the plants perished before assays could be made in midsummer.

Two small trees of red maple growing in the greenhouse were ringed on March 24. After one month somewhat more of the vitamin was found in the bark below than above the ring. After 4 months, however, there was five times as much vitamin above as below the ring. The values after four months were as follows: bark above the ring, 5.7 and 3.5; and below the ring, 0.8 and 0.9 mg. per kg. Two basswood trees about three inches in diameter were ringed on March 15. At the time of ringing the bark assayed 1.0 and 0.9 mg. per kg. of fresh tissues. About four months later (on July 12) the determinations were as follows: bark above the ring, 5.4 and 4.2; and below the ring, 0.8 and 1.0 mg. per kg.

From the data on white pine and red maple it seems that a short time after ringing a tree in early spring, the amount of vitamin per unit of fresh bark is depleted less rapidly below than above the ring. In contrast to this temporary condition the data obtained with ringed basswood and red maple suggest that after a longer time impedance of transport downward from the leaves may result in accumulation of vitamin in the bark just above the ring.

Bonner (1940) has reported the inhibition of transport of thiamine across zones of tomato stem which had been killed by steam. Presumably the supplies below the ring in a girdled tree are finally almost completely exhausted. Possibly girdled trees may ultimately die not alone from food starvation but from extreme depletion of vitamins necessary for the roots.

SUMMARY

The approximate thiamine activity as indicated by the *Phycomyces* assay of buds, leaves, and bark of some common American trees was studied at different seasons of the year.

Significant differences in stimulation of *Phycomyces* by extracts of tissues were found among individuals of white pine, between the needles and buds, and at different levels in the bark of these trees.

Both buds and leaves of sugar maple and white oak inhibited growth of *Phycomyces* in nutrient solutions to which had been supplied amounts of synthetic thiamine more than enough for normal growth of the fungus. Crude extracts from such species cannot be assayed for thiamine with the simplified method. Extracts from red maple and many other trees had no such inhibitory influence.

The buds and leaves of several species of deciduous trees sampled at different seasons of the year showed varying degrees of effectiveness in promoting growth of *Phycomyces*.

In the bark of young trees girdled in early spring, less thiamine activity was found above than below the girdle after 4 to 6 weeks. After about four months, however, substances showing thiamine activity accumulated in the bark above the ring probably as a result of inhibited translocation downward from the leaves. It seems possible that girdled trees may ultimately die from vitamin starvation of their root systems.

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AN ISOLATED COLONY OF PLANTS ON A GLACIER-CLAD MOUNTAIN

WILLIAM S. COOPER

In late July, 1940, Mr. and Mrs. Bradford Washburn and three companions made the first ascent of Mt. Bertha, a difficult peak of the Fairweather Range in the Glacier Bay National Monument, Alaska. In the course of their climb they found patches of vegetation growing in such inhospitable situations that they made collections and later submitted them to me. The set comprises 15 species. Only four are first reports for the Glacier Bay National Monument,[1] but because of the conditions under which they grew, and the occurrence of certain species far from their previously known ranges, the collection is of very special interest.

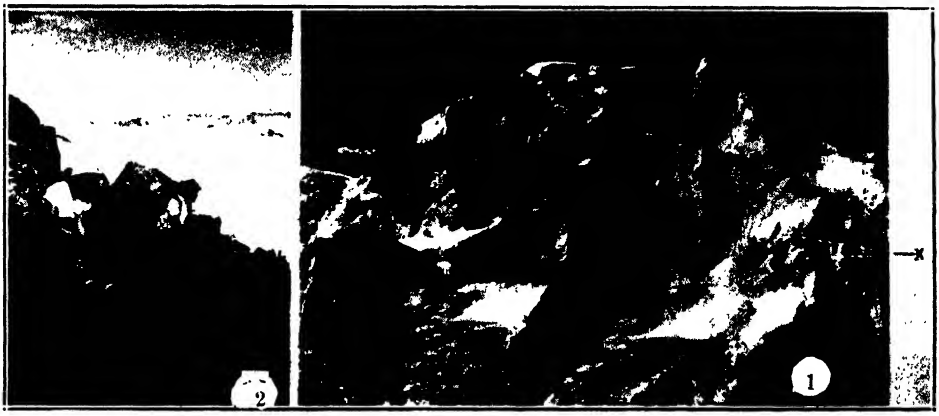

FIG. 1. Mt. Bertha from the southeast. Crosses mark some of the higher spots where collections were made. Aerial photograph by Bradford Washburn. FIG. 2. A patch of vegetation on the southeast ridge of Mt. Bertha at 5000 ft. *Lutkea pectinata* and *Lupinus nootkatensis* are distinguishable. Photograph by Bradford Washburn.

The altitude of Mt. Bertha is 10,182 ft. It constitutes a bastion projecting eastward from the mass of Mt. Crillon, 2500 ft. higher, which stands upon the main divide between Glacier Bay and the ocean. The mountain itself is largely sheathed with ice and snow (fig. 1). Everywhere around it are glacier-clad peaks; the intervening valleys are occupied by névé and ice-streams. Particularly noteworthy is the Brady Ice Field, 30 miles long and 5–10 miles wide, which partially surrounds Mt. Bertha on the east and south. The

[1] Previous lists for the area have been published by the writer: The seed-plants and ferns of the Glacier Bay National Monument, Alaska. Bull. Torrey Club 57: 327–338. 1931. Additions to the flora of the Glacier Bay National Monument, Alaska, 1935–1936. Bull. Torrey Club 66: 453–456. 1939.

shortest distances to lowlands bare of snow in summer are westward to the vicinity of Crillon Lake, 14 miles, and northward to Johns Hopkins Fiord, 12 miles. Very high mountains here intervene, and the latter inlet became free of its trunk glacier only within the last half century. To Geikie Inlet, tributary to lower Glacier Bay, the distance is 17 miles. Two centuries ago, when the basin of Glacier Bay was completely filled with ice,[2] distance to unglaciated areas northward and eastward was 40-50 miles.

The plants were collected along the crest of an eastward-projecting spur, mainly on the south exposure, at altitudes ranging from 4100 to 6000 ft. They grew on small patches of gravel between ledges. Some of the higher localities where specimens were gathered are indicated by crosses on figure 1. At the time of collection the rocks in this vicinity were somewhat barer than is shown in the picture, which was taken two weeks later, after a heavy fall of snow. The growing season here is six weeks at the most.

The list of species collected is given below, and comments concerning taxonomy and individual ecology are added. Specimens have been deposited in the herbarium of the University of Minnesota.

LYCOPODIUM SELAGO L. Plants of normal size and character. A typical member of the heath-mat community which is widespread above timberline and occasional below, even to sea-level.

SILENE ACAULIS L. Normal. A common pioneer on gravels, mainly above timberline but occasional even at sea-level.

SAXIFRAGA BRONCHIALIS L. Moderately dwarfed. This and the two following inhabit rock crevices and similar habitats below timberline. They are not characteristically alpine.

SAXIFRAGA PUNCTATA L. One-third to one-half the average height at lower altitudes. First report for National Monument.

SAXIFRAGA TRICUSPIDATA Rottb. Normal size for Alaska specimens.

LUTKEA PECTINATA (Pursh) Kuntze. A characteristic member of the heath mat. Basal leaves small, short-stalked, in crowded rosettes; stem leaves abruptly reduced, very small.

LUPINUS NOOTKATENSIS Donn. Very much dwarfed. A typical lowland plant, abundant in various meadow communities, where it grows to a height of at least 3 feet. The highest point where this species was found was at 5000 ft.

EPILOBIUM LATIFOLIUM L. Dwarfed. Leaves and flowers one-half to two-thirds the size of lowland specimens.

PHYLLODOCE GLANDULIFLORA (Hook.) Coville. Normal except that leafy stems are very short. A characteristic heath-mat species.

POLEMONIUM HUMILE Willd. Somewhat dwarfed, leaflets smaller than

[2] Cooper, W. S. The problem of Glacier Bay, Alaska: a study of glacier variations. Geogr. Rev. 27: 37–62. 1937. A map accompanying this paper shows the location and surroundings of Mt. Bertha.

average, pubescence more abundant, especially in inflorescence. An apparently delicate plant of the subalpine forest. First report for National Monument.

CAMPANULA LASIOCARPA Cham. Plant normal in height (½–2 inches); flower larger than normal. A characteristic alpine species.

ANTENNARIA ISOLEPIS Greene.

ANTENNARIA MEDIA Greene.[3] In studying the small set of three individuals of Antennaria, my first assumption was that they were all *A. borealis* Greene, which I have collected frequently at low altitudes around Glacier Bay and Prince William Sound. It turned out, however, that according to the treatments by Greene[4] and Malte[5] (the only pertinent ones) two species are represented, neither being *A. borealis*. These two, moreover, have ranges distinct and far-separated so far as known, both very distant from Glacier Bay.

A. media is a cordilleran high-mountain species ranging from the central Sierras north to southern British Columbia and eastward to Glacier Park and the Wasatch Mountains. The nearest known stations to Glacier Bay are Mt. Arrowsmith, Vancouver Island, and Griffin Lake, near Revelstoke, 800 and 900 miles distant respectively. The specimens match very perfectly the abundant material in the herbarium of the University of Minnesota. They are pistillate plants, as is also the single specimen of *A. isolepis*.

A. isolepis ranges "from Labrador and northern Quebec to Keewatin and perhaps farther west."[6] The single individual checks perfectly with Malte's description and with two specimens from the Torngat region from collections cited by Malte. The nearest known authentic localities are near the west coast of Hudson Bay, 1400 miles distant from Mt. Bertha. Polunin,[6] however, mentions a somewhat doubtful specimen from Mt. Selwyn, B.C. (lat. 56° N, long. 123° 30′ W), which is only 500 miles from Mt. Bertha.

It is of course true that the regions lying between Glacier Bay and the known ranges of the two species have been very inadequately explored. It is rather startling, however, that a cordilleran and an arctic species should first be detected in the far northwest perched in close company upon a lofty ridge surrounded by miles of glaciers; in a region, moreover, that has enjoyed a fair amount of botanical exploration, at least at low altitudes.

HIERACIUM GRACILE var. MINIMUM A. Nelson. Two inches in height; normal for the alpine variety.

SOLIDAGO MULTIRADIATA var. SCOPULORUM A. Gray. Two inches in height; normal for the alpine variety.

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[3] I wish to thank Drs. F. K. Butters, C. W. Sharsmith, and J. W. Moore for assistance in identifying this interesting pair of species.

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The collection is admittedly incomplete, and therefore an adequate characterization of the plant community is impossible. Mr. Washburn makes one suggestion, however, that is significant. He writes that "the flowers occurred in little patches of anywhere from two or three feet to fifteen or twenty feet long. Some of these patches were typical 'turf-banked terraces'; others were sunny spots sheltered by surrounding loose rocks or ledges" (see figure 2). The turf was from two to three inches thick. This suggests the presence of minute samples of the characteristic alpine mat of southeastern Alaska. Three of the species collected are typical members of that community: *Phyllodoce glanduliflora, Lutkea pectinata,* and *Lycopodium selago.* Four others are definitely arctic-alpine: *Silene acaulis, Campanula lasiocarpa,* and the two Antennarias. The remaining eight are not characteristically alpine. Two species, *Lupinus nootkatensis* and *Polemonium humile*, the first ordinarily found in sea-level meadows, the second in moist sheltered spots in the subalpine zone, seem particularly out of place among these snowbanks. Although dwarfed, they appear otherwise normal and healthy. In general, the subalpines are more or less dwarfed while the characteristic alpines are normal in size.

Two questions present themselves: How did these plants reach their present position, and how long have they been there? Six species, *Lycopodium selago, Epilobium latifolium, Hieracium gracile, Solidago multiradiata,* and the Antennarias, have disseminules more or less well fitted for transportation by wind. These might perhaps have been wafted over the 15 miles or more of ice fields separating them from possible sources. The others have small seeds, except *Lupinus nootkatensis* which has large, heavy ones. Wind transportation of small seeds across the névé of the Brady Ice Field, perhaps by repeated jumps, with a final lift to the slopes of Mt. Bertha by violent ascending air currents, is conceivable. The entire trip, however, would have to be completed between the time of seed ripening and the onset of winter snow; otherwise, in this region of tremendous snowfall, hopeless burial would take place.

If the barriers to migration appear formidable today, they were even more so two centuries ago, when the whole area tributary to Glacier Bay was buried under ice. The gathering of this diverse assemblage of species in so limited and isolated a spot, under present conditions or worse, even granting a century or two for the process, and particularly in view of the presence of a turf mat, the development of which is a slow process, puts a serious strain upon the imagination.

A much more easily tenable hypothesis is that there has been a continuous plant population high on the slopes of the mountain for a very long time, and that it arrived during a period when glaciation was considerably less extensive than now, when easy migration routes led from the lowlands to the

upper slopes, and when areas suitable for alpine vegetation were more widespread in the immediate vicinity.

A period of this character has been demonstrated for the Glacier Bay region. The glaciers in their retreat are still uncovering the remains of forests which existed when the ice fields were considerably less expanded than they are today.[7] The cause of glacier contraction was presumably the onset of climatic conditions relatively unfavorable to snow precipitation and preservation—of comparative warmth or dryness or both. It was followed by return to conditions favoring glacier expansion, which have persisted into recent time.

This sequence agrees with the post-Pleistocene climatic history that has been tentatively worked out for northern North America in general and for northern Europe as well. The scheme, whose formulation is due principally to von Post, postulates a period of comparative warmth and dryness in mid-post-Pleistocene time followed by a return to conditions moderately cool and moist. There is North American evidence for it from a number of independent fields, which has been summarized by several writers.[8] The history of the plant population of Mt. Bertha here suggested may be fitted into this scheme with no contradiction of known facts.

It is natural to assume that other similar isolated colonies exist. Mr. Washburn, who has seen more of the higher parts of the Fairweather Range than any other person, has found none there. From the St. Elias Range, however, he reports "small patches of flowers very similar to these about thirty miles from timber on a little rock island [in the ice fields]." Dr. F. K. Butters has seen patches of alpine plants inhabiting high ridges in the Selkirks surrounded by miles of snow fields. A particularly interesting case is the finding of *Loiseleuria procumbens* in such a situation—this being its only recorded occurrence in the Selkirk Mountains. It is quite possible that directed search would show that such isolated colonies are by no means rare among glacier-clad mountains.

A final suggestion: if these colonies have held on in isolation through several thousand years of glacial rigor, is it not equally believable that similar ones may have survived on nunataks through one or even more of the continental ice floods of the Pleistocene?

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A NEW TETRAPLOID WHEATGRASS FROM NEVADA

JOSEPH H. ROBERTSON AND LLOYD WEAVER[1]

A group of extremely large plants of *Agropyron spicatum* (Pursh) Scribn. and Smith, has been observed growing near the Paradise Valley Substation, Intermountain Forest and Range Experiment Station, four-and-one-half miles west of the town of Paradise Valley in Humboldt County, Nevada. These plants were first noticed during the summer of 1940. The associated species are all of normal stature and include the following: *Artemisia tridentata, Purshia tridentata, Elymus condensatus, Bromus tectorum, Stipa*

FIG. 1. A tetraploid plant (left) and a normal plant (in hand) of *Agropyron spicatum*, and a clump of *Elymus condensatus* (right). Paradise Valley, Nevada, October 1940. U. S. Forest Service Photo.

thurberiana, Agropyron spicatum, Astragalus whitedii, and *Balsamorhiza sagittata.* This community occupies an irregular area of some 10 square rods on very fine sandy loam sloping gently to the southeast toward Lamance Creek.

So robust are the large plants of *Agropyron* that casual observation failed to distinguish them from the giant wild-rye (*Elymus condensatus*) preva-

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lent in the community (fig. 1). In nearly all macroscopic characters the giant form is approximately twice as large as plants of the normal variety growing on the same site (table 1).

TABLE 1

Comparative measurements[a] of gross characters of normal and giant varieties of Agropyron spicatum near Paradise Valley, Nevada

Character	Normal	Giant
Diameter of bunch, range	15–30 cm.	8–74 cm.
Height of culms, average	60 cm.	120 cm.
Leaf width,[b] average	2.8 mm.	7.0 mm.
Leaf length,[c] average	16.5 cm.	34.5 cm.
Ratio: Leaf length to width	58.9 : 1	49.3 : 1
Length of spike, average	11.5 cm.	18.7 cm.
Length of spike, range	5–18 cm.	10–23 cm.
Length of spikelet,[d] average	16.8 mm.	23.6 mm.
Dry weight,[e] herbage	35 gm.	77 gm.
Percentage seed germination, 1941	24	0

[a] Although no basis for statistical comparison is available, these data were obtained from 5 representative plants of each form, and are believed to be unbiased and to represent real differences.

[b] Widest portion of third culm leaf below the spike.

[c] Same leaf as the preceding.

[d] Spikelets at center of spike; awns excluded.

[e] Bunches of equal diameter, 15 cm.

Despite its greater size and yield per plant, the value of the giant variety as a range forage plant is not established. No viable seed was produced by either the giant or the normal variety in 1940. In spite of apparently favorable rainfall in 1941, only seed of poor quality was obtained from the normal *Agropyron spicatum*, while that from the adjacent giant plants was entirely nonviable (table 1). It does spread by short, stout rhizomes, and the presence of bunches of various diameters is evidence that occasional crops of viable seed have been cast.

In October 1940, a bunch was divided and the clons potted in the greenhouse at Ogden, Utah. Some of the greenhouse material was sent in March 1941 to the Osborn Botanical Laboratory of Yale University for study. This study consisted of chromosome counts of mitotic figures in root tips and a comparison of sizes of stomata and epidermal cells of the leaves.

Root tips of the giant *Agropyron* were fixed in CRAF solution, embedded in paraffin, sectioned, and stained by means of the Feulgen reaction.

Mitotic figures were not numerous in the root tips, but enough polar views of metaphase plates were present to make possible eight chromosome counts. In addition, a number of figures showed an indefinite number of chromosomes; in all cases more than 14 chromosomes were distinguishable. Of the eight cells mentioned above, one clearly contained 28 chromosomes (fig. 2), and the other seven showed from 21 to 25 with the probability of

additional ones in places where chromosomes were coiled about one another in such a way as to make definite identification of individuals impossible.

Peto[2] recognized the chromosome number of *A. spicatum* as $2n = 14$. The occurrence of 28 somatic chromosomes in root-tip cells of the large plant, supplemented by identification of the form as *A. spicatum* at the Bureau of Plant Industry in Washington, D. C. (U. S. Nat. Herb. No. 90907), as well as by Dr. Bassett Maguire of the herbarium at the Utah State Agricultural College, Logan, Utah, seems sufficient to establish the fact that the giant is a tetraploid form of *A. spicatum*.

Blakeslee and Warmke[3] and others have used gross and semi-microscopic morphological characters such as larger pollen grains in $4n$ flowers, larger

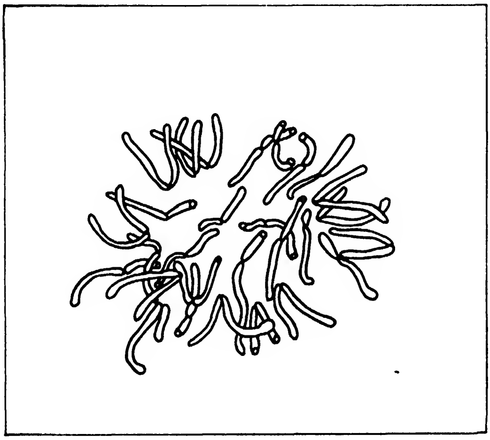

FIG. 2. Polar view of mitotic metaphase from a root tip of the giant *Agropyron spicatum*. Approximately × 2500.

$4n$ seeds, larger stomata, leaves increased in width and thickness, larger size of floral parts, and shorter, stouter fruits as criteria of polyploids. In this species, however, no significant difference was found in size of stomata of the tetraploid as compared with the diploid. An examination of table 1 shows a pronounced decrease in ratio of length to width of the tetraploid leaves. Spikelets are larger than on the diploid form, and the entire inflorescence is appreciably larger in the giant form.

Interspecific hybridization as a means of origin of new *Agropyron* species is discussed by Peto (1930), who cites instances of natural hybridization between *A. cristatum* and *A. repens* var. *glaucescens* in Russia and between *A. junceum* and *A. repens* in Denmark. The hybrids mentioned were similar in form to entirely different species of the genus. It is barely possible that the giant form here discussed is such a hybrid. This hypothesis is supported

[2] Cytological studies in the genus *Agropyron*. Canad. Jour. Res. 3: 428–448. 1930.

[3] Size of seed and other criteria of polyploids. Science 88: 440. 1938.

by the limited distribution, scant seed production, and the frequent presence in the giant plants of short rhizomes which occur only rarely in *A. spicatum*. That the gigantism may be an extreme case of hybrid vigor, however, seems extremely improbable. *A. repens* is the only 42-chromosome member of the genus believed to be present in the locality. Fertilization of 7-chromosome *A. spicatum* eggs by 21-chromosome pollen of *A. repens* or vice versa would result in a hybrid with 28 chromosomes, but it seems hardly possible that such a hybrid would possess only one important characteristic of *A. repens*, namely the presence of more vigorous rhizomes, while all other parts, though larger, were distinctly and unmistakably those of the other parent.

In considering the lack of definite evidence for hybridization as compared with the occurrence of distinctly tetraploid morphological characters and the double number of chromosomes, it seems most likely that this giant wheatgrass is a tetraploid form of *A. spicatum*.

U. S. Department of Agriculture, Forest Service
Ogden, Utah

BULLETIN OF THE TORREY BOTANICAL CLUB
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A NEW GENUS OF RUBIACEAE FROM MEXICO

MAXIMINO MARTÍNEZ

In September, 1941, Miss Marian Storm, author and enthusiastic admirer of the Mexican flora, brought to my attention a shrub which grew in the region of Uruapan, Michoacán. This plant, with its brilliant scarlet-red flowers has long been a favorite of the people of the region and is locally known as *Ayuque*. Because of the condition of the original material, no satisfactory identification of it could be made. A former student of mine, Sr. Emilio Zamudio, was therefore commissioned to collect material sufficient for its further study. The specimens were refractory and did not yield characteristic examples with the usual methods of preparation. As a consequence, it was necessary to devise special techniques for their preservation. Ample material, preserved in formalin, was also made available.

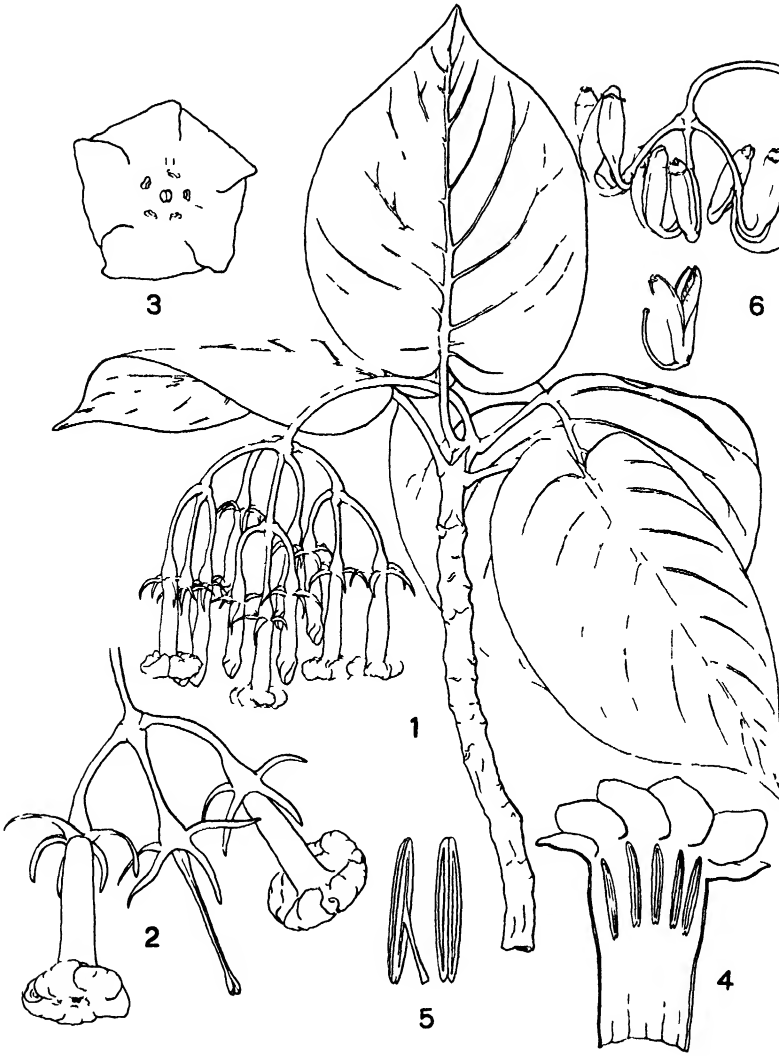
Collections of both flowering and fruiting material, made over a period of several months, lead me to the conclusion that the *Ayuque* represents not only a hitherto undescribed species but also a new genus of the Rubiaceae, closely related to *Cosmibuena* and *Blepharidium*. The following new genus and species are therefore proposed:

Balmea Martinez, gen. nov. Flores penduli; sepala 5, lobis linearibus, subpersistentibus; corollae tubus fere cylindricus, lobis ovatis; stamina infra faucem corollae inserta, filamentis planis, antheris dorsifixis, fusiformibus; capsula erecta; seminis ala indivisa.

TYPUS: species una mexicana, *Balmea stormae*.

Balmea stormae Martinez, sp. nov. Frutex omnino glaber, 5 (4–7) m. altus, ramis ascendentibus, basi 15–20 cm. diam.; lignum fere albidum, cortice 5–10 mm., epidermide chartacea, lutescenti, paullo violacea; ramuli tortuosi, glabri, fulgentes, cinerascentes; folia petiolata, opposita, apice ramulorum conferta, stipulata, crassiuscula, fulgida, flexibilis, siccitate opaca, aspera, laevis; lamina foliorum ovata, apice breviter acuminata, base rotundata vel plus minusve late cordata, 9–13 cm. longa, 6.5–11 cm. lata, integra, utrinque glabra; petiolus 2.5–4 cm. longus, supra canaliculatus, ruber, subtus convexus, viridis, tandem violaceus; nervi claro virides, interdum rubro-violacei, basi subtus subelevati; stipulae interpetiolares, ovato-acuminatae, crassiusculae, interdum papyraceae, caducae, 10 mm. longae; inflorescentia terminalis, cymosocorymbosa, pendula, laxa, multiflora (usque ad 13-flora), trifurcata vel bifurcata, pedunculo communi 5.5 cm. longo; flores hermaphroditi, nocte fragrantes; corollae tubus fere cylindricus, glaber, 22–28 mm. longus, basi leviter attenuatus, rubro-coccineus, dein purpureus, limbo 5-fido, stricte contorto, lobis ovatis vel rotundatis, reflexis, 10 mm. longis; sepala 5, linearia, expansa, luteo-viridia, subpersistentia; stamina 5, cum corollae lobis alternantia, antheris bilocularibus, subsessilibus, introrsis, longitudinaliter dehiscentibus, filamentis liberis, brevibus, planis, 4 mm.

longis; ovarium oblongum, inferum, biloculare, sepalis coronatum, ovulis numerosis; stylus crassiusculus, glaber, rubro-violaceus, 20–23 mm. longus, 2-sulcatus, apice clavatus, bifidus; capsula ovali-oblonga, bisulcata, bival-



FIGS. 1–6. *Balmea stormae.* FIG. 1. Flowering branch FIGS. 2, 3, 4. Corolla. FIG. 5. Stamens. FIG. 6. Fruit.

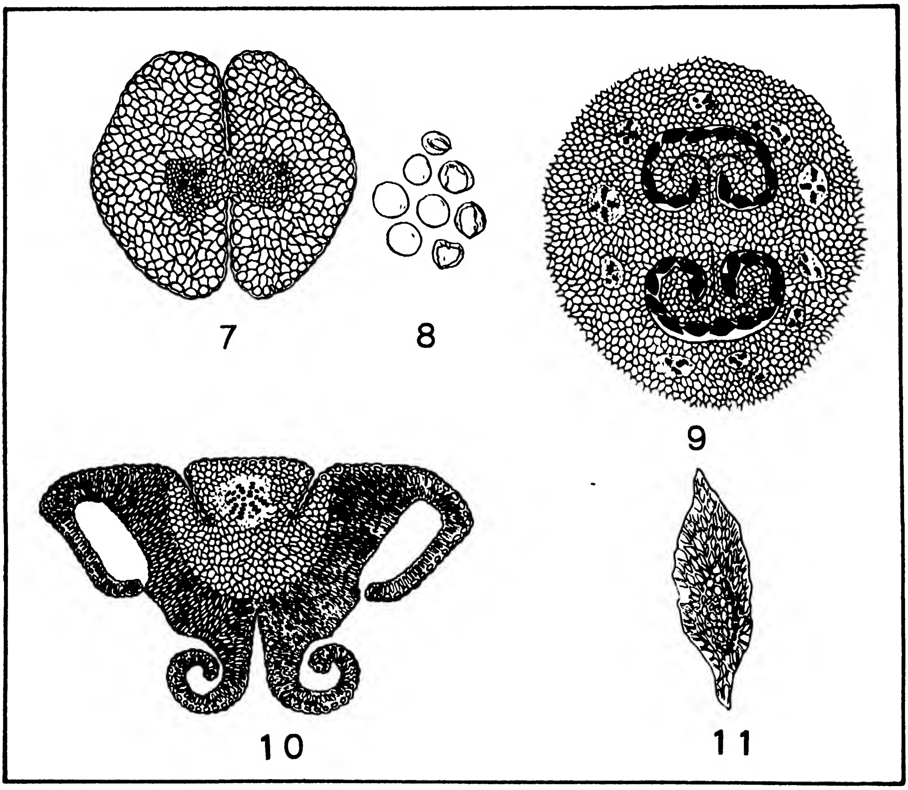
vata, chartacea, junior pendula, maturitate erecta; placentae longitudinales, 2-lamellatae, septo adnatae; semina numerosa, parva, ascendentia, imbricata, alata, ala reticulata, indivisa, 3.5–4 mm. longa.

Floret Augustus–October; semina matura in Decembri vidi.

Habitat in Palo Verde, Pedregales de Jicalán et San Pedro, prope Uruapan, MICHOACÁN, MEXICO.

Typus (*Martínez 3400*) in Herb. Instituto de Biologia, Mexico; Isotypi in Herb. Smithsonian Inst., Gray, Field Mus., Arnold Arboretum, Missouri Bot. Gard., New York Bot. Gard., Univer. Calif., Kew.

A shrub 4–7 meters high, glabrous throughout, branched from below, the individual stems basally 15–20 cm. in diam. Bark smooth, 5–10 mm. thick, greenish purple, peeling off in irregular thin shreds; wood hard, whitish. Branchlets irregular, grayish, sublustrous, showing very clearly the marks



FIGS. 7–11. *Balmea stormae*. FIG. 7. Cross section of the style. FIG. 8. Pollen. FIG. 9. Cross section of the interior of the ovary. FIG. 10. Cross section of the anther. FIG. 11. Seed.

of the petioles. Leaves clustered at the ends of the branches, petiolate, stipulate, opposite, broadly ovate and shortly acuminate, the base obtuse or slightly cordate, entire and deciduous, somewhat fleshy, smooth and flexible, light green and lustrous, the nerves slightly prominent only at the base and underneath, the blade 9–13 cm. long, 6–11 cm. wide, the petiole canaliculate above, where it is red or purple, below convex and greenish; when the weather is exceptionally dry the color spreads to the whole petiole and to the nerves. When dry the leaves turn dull, thinner and somewhat rough. Stipules interpetiolate, triangular, acutish, reddish, slightly fleshy when young,

papyraceous and yellowish when old, 10 mm. long, caducous with the growth of the leaves, leaving a clear mark on the branchlet. Inflorescence terminal, in corymbose cymes, pendulous, in groups of 9–13 flowers, the main peduncle bi- or trifurcate. After the corollas fall and with the ripening of the fruit, the pedicels twist upwards and the capsules remain erect. The common peduncle is 5–5 cm. long and the pedicels 2 cm. long.

Flowers hermaphrodite, gamopetalous, scarlet-red, tinged with purple and, when fully open, dark purple. They are sweet-scented at night. Calyx with 5 linear sepals, yellowish or greenish, 8–10 mm. long, persisting some time after the falling of the corolla. Corolla soon deciduous, its tube 22–28 mm. long, subcylindric, a little narrowed at the base, at first red but soon turning purplish; lobes 5, subovate, entire, imbricate, overlapping to the right (when the lobes open they become reflexed and the overlapping appears to be to the left). Stamens 5, alternate, inserted near the mouth of the tube. Anthers fusiform, 9–10 mm., included, the tips visible but not exerted, bilocular and valvate. Filaments short and flat, 4 mm. long. Style the same color as the corolla, 20–23 mm. long, marked with two opposite furrows. Stigma bilamellate. Ovary inferior, oblong, marked on each side with a suture, bilocular, with the placenta adnate to the septum; ovules numerous. Capsule erect, oval-oblong, 23–28 mm. long, opening at the apex in two follicle-like portions; epicarp papyraceous; seeds numerous, imbricated lengthwise, with a reticulate wing 3.5–4 mm. long.

The shrub flowers from August to October and the capsules are ripe from December to January. Habitat in Palo Verde, the Pedregales de Jicalán and San Pedro, near Uruapan, Michoacán; in dry stony places.

The genus is named in honor of Professor Juan Balme, able student of ornamental plants (to which the *Ayuque* is certainly an addition), and the species in honor of Miss Marian Storm, an enthusiastic collaborator in various of my botanical works.

I also wish to express my appreciation for kind assistance to Dr. F. Miranda, P. C. Standley, C. V. Morton and to Sr. Manuel Ornelas C. to whom I am indebted for the illustrations.

Morelia 61
Mexico, D. F.

BULLETIN OF THE TORREY BOTANICAL CLUB
69(6): 442–444 JUNE, 1942

A NEW GENUS OF THE ANACARDIACEAE FROM COLOMBIA[1]

FRED A. BARKLEY

The author is indebted to Dr. E. P. Killip for the specimen on which this genus is based. It was sent with the notation that it might prove to be a new genus and this was borne out by a study of the specimen.

This tree grows in the dense forests in the mountains of Colombia. The pith is thick, similar to that of *Rhus*. The branches are densely puberulent, as are the leaves and inflorescences. The flowers have five persistent sepals, five caducous petals, five stamens, a prominent disk, and a tricarpellary pistil. The fruit is outstanding, being a samara-like drupe remindful of *Pseudosmodingium* but bearing long violet-colored hairs similar to that of the fruit of *Actinocheita*, but limited to the margins of the fruit much as in *Heliocarpus.*

It is known only from the type collection.

Ochoterenaea[2] Barkley, gen. nov. Arbor; ramis crassis; foliis imparipinnatis; floribus multis in thyrsis ad terminos ramorum brevium lateralium; sepalis 5 persistentibus, petalis staminibusque 5, pistillo tricarpellato, carpello unico fertili; fructu planissimo, drupa, glabro margine longe pilosa excepta.

Tree; leaves imparipinnate; flowers many in thyrsi at the ends of short lateral branches; sepals 5 persistent, petals and stamens 5, disk prominent, pistil tricarpellary with one fertile carpel; fruit a very flattened drupe long-pilose along the margin.

TYPE SPECIES: **Ochoterenaea colombiana** Barkley, sp. nov. Arbor ad 20 m.; ramis crassis; foliis imparipinnatis, foliolis 11–13, lanceolatis, integerrimis; ramis, foliis, thyrsisque pubescentibus; floribus multis in thyrsis ad terminos ramorum brevium lateralium; sepalis 5 persistentibus, petalis staminibusque 5, pistillo tricarpellato, carpello unico fertili; fructu planissimo drupa, glabro margine longe pilosa excepta.

Tree to 20 meters; branches thick; leaves about 45 cm. long, imparipinnate with 11–13 leaflets; leaflets entire, lanceolate, 11–18 cm. long, 4.5–6 cm. broad; branches, leaves and inflorescence densely puberulent; flowers many in thyrsi at the ends of short lateral branches; sepals 5, persistent, deltoid-ovate; petals 5, caducous, lanceolate; stamens 5; pistil tricarpellary with two abortive carpels; fruit a much flattened drupe, glabrous except for a region on and near the margin which is long pilose with violet hairs and except for a very few glandular hairs mostly limited to the margin.

TYPE: *Killip 34772,* dense forest, Rio Digua Valley, between La Elsa and Rio Blanco, department El Valle, Colombia, altitude 725 meters, April 2–5, 1939, in U. S. National Herbarium No. 1771563.

[1] The author wishes to acknowledge assistance from grants from the Penrose fund by the Research Committee of the American Philosophical Society in his studies on the Anacardiaceae, of which this is a part.

[2] Named in honor of Dr. I. Ochoterena, Director of the Biological Institute of Mexico.

FIG. 1. Type specimen of *Ochoterenaea colombiana* Barkley.

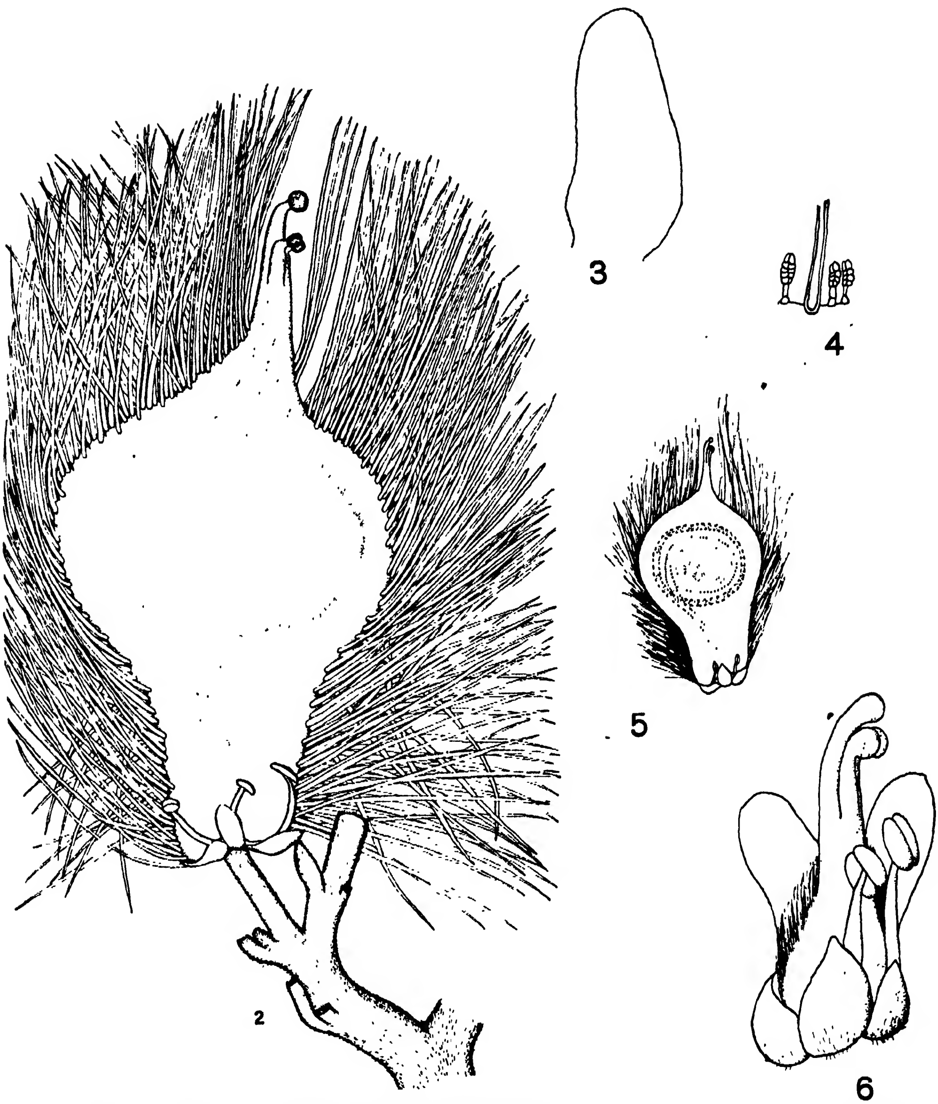


FIG. 2. The fruit of *Ochoterenaea colombiana* with the upper portion of the flowering branch. Note bract scars, persistent sepals, prominent disk, and the marginal position of the pubescence. × 9. FIG. 3. A petal from *O. colombiana.* ×18. FIG. 4. A portion of the margin of the fruit showing glandular hairs. × 120. FIG. 5. Dissection of the fruit of *O. colombiana* showing the endocarp, and orientation of the ovule and embryo. × 4. FIG. 6. A flower of *O. colombiana* with three petals and three stamens removed. × 18.

Specimens are deposited or will be distributed as follows: Ciencias Naturales, Bogota; British Museum, London; U. S. National Herbarium, Washington (two sheets); and in the author's Herbarium.

DEPARTMENT OF BOTANY, MONTANA STATE UNIVERSITY
MISSOULA, MONTANA

BULLETIN OF THE TORREY BOTANICAL CLUB

69(6): 445–460 JUNE, 1942

NEW AND CRITICAL EUPHORBIACEAE CHIEFLY FROM THE SOUTHEASTERN UNITED STATES

LEON CROIZAT

Three new species of *Croton* L. and one of *Manihot* Mill. are recorded in this paper for the flora of south-western Texas. A fourth species of *Croton* L. is described from Sonora, Mexico. Lastly, a summary review is given of *Tetracoccus* Parry under which are published three new subgenera and two new combinations. The Latin diagnoses required for these publications are contained in an appendix at the end of this contribution.

CROTON L.

Croton parksii Croizat, sp. nov. A shrub, apparently dioecious. *Innovations* stellate-tomentose, dull yellow to olivish, tardily glabrescent. *Leaves* reminiscent of the foliage of *C. capitatus* Michx., 2.5–6 cm. long, 1–2 cm. broad, tomentose throughout, grayish underneath, olivish to brownish above, mostly elliptic, more or less short-rounded at the tip, rounded at the base, with about 6–8 pairs of ascending primaries; petiole not over 1 cm. long, slender, tomentose, glands at the lower face of the blade near the insertion of the petiole 2, inconspicuous, basal stipules obscure. *Inflorescence* of coarctate terminal and lateral thickly flowered axes, the ♂ subspicate, sometimes tending to be racemose, the ♀ capitate. *Perianth* ♂ about 8 mm. wide, tomentose, delicate, on a pedicel not over 2 mm. long; petals none; lobes (sepals) 6, sometimes 5, ovate to obovate, about 3 mm. long, 2 mm. wide, heavily ciliate-lanulose, thinly venulose toward the center and there greenish; androecium of about 10–15 stamens inclosed within a disc of 5 conspicuous glands each approximately set at the base of a lobe. *Perianth* ♀ about 5 mm. wide, becoming about 10 mm. wide under a young fruit, tomentose except at the disc and often at the inner face of the lobes, pedicel stoutish about 2 mm. long; petals none; lobes not delicate, triangular-elliptic, about 2 mm. long; disc thin, shallowly cup-shaped under the ovary and slightly broader than it; ovary at first appearing heavily tomentose, soon developing scattered and conspicuous submuricate processes on the epicarp, these heavily coated with whitish stellate tomentum, about 2–3 mm. large in anthesis; styles 3, free, each 2 mm. long, somewhat irregularly 2–3 times cleft, the stylar laciniae forming together a closely matted and involute cluster partly immersed within the thick tomentum of the epicarp, somewhat carnose and brownish where free; fruit capsular, 3-coccous, about 8–9 mm. long and as many broad, globose; seed more or less globular, large for a species in this affinity, about 7 mm. long, 6 mm. broad, the caruncle contiguous with the hilum and produced into a short process just above it, broadly spreading to cover the entire upper end of the seed, the aril smoothish, coarsely mottled by spots and streaks of black and whitish on a background of brown and hazel hues, the raphe thin but conspicuous, the chalaza located on the ventral face of the testa within a small depression, the testa (scraped free from the aril) apparently smooth; columella after dehiscence about 7 mm. long, trans-

versely expanded to about 4 mm.; dehisced cells about 9 mm. long, the endocarp firm, horny, the epicarp fairly thin, finely veined at the inner side when free from the endocarp.

HOLOTYPE: *H. B. Parks s.n.*, Sutherland Springs, Texas, Oct. 1936 (♀ specimen). Flowers ♂ described from *H. B. Parks s.n.*, same locality and date (Gray Herbarium).

The following collections belong here: (1) *H. B. Parks s.n.*, Ingleside, San Patricio Co., Texas, Oct. 1936; (2) *H. B. Parks s.n.*, Flour Bluff, Nueces Co., Texas, Oct. 1936; (3) *V. L. Cory s.n.*, Padre Island 26 miles south of Port Aransas, Texas, Nov. 1940.

A strong species easily distinguished from every other in its affinity but nearest to *C. texensis* Muell.-Arg., from which it differs in the larger capsule and seed, in the outstanding murication of the epicarp, in the more heavily tomentose leaves. Specimens past fruiting are identified by the stoutness and breadth of the columella. *Croton muricatus* Nutt., as shown by the classic specimens in the herbarium of the New York Botanical Garden, is not this species but a synonym of *C. texensis.*

Croton parksii belongs to *Croton* sect. *Drepadenium* Muell.-Arg., which is one of the few natural groups in the classification of *Croton* as treated by Mueller. The distribution of this section has unusual interest. Its type, *C. punctatus* Jacq., ranges from Venezuela to Virginia as a plant of the sea-shore. Other species (*C. texensis* Muell.-Arg., *C. californicus* Muell.-Arg. and the segregates in its vicinity, *C. neomexicanus* Muell.-Arg., *C. gracilis* H.B.K., *C. luteo-virens* Woot. & Standl., *C. parksii* Croiz.) range inland from Texas to northern Mexico and California. This distribution suggests that the ancestral forms in this complex were plants of the shore, as *C. punctatus* still is, which speciated adapting themselves to changed surroundings when the waters withdrew or evaporated in land-locked basins in parts of the ancestral range. The background of this evolution is almost certainly Tertiary if not early Tertiary, there being no lack of evidence that the generic lines of the Euphorbiaceae were drawn at the closing of the Cretaceous. Distributions and speciations of the same order as those characteristic of the *C. punctatus* group are in evidence among certain euphorbiaceous aggregates in Argentina and Central Asia.

Croton coryi Croizat, sp. nov. A shrubby perennial. *Innovations* thickly and coarsely hispid-tomentose, grayish to olivish, tardily glabrescent. *Leaves* verticillate at the apex of the branchlets, 3.5–5 cm. long, 1.5–2 cm. broad, elliptic-lanceolate, round-acuminate at the tip, rounded to subtruncate at the base, coarsely tomentose to hispid-pannose, olive nearly the same on both faces, with about 7–8 pairs of sharply ascending primaries scarcely visible under the indumentum; petiole 1.5–2.5 cm. long, hispid, glands apparently none. *Inflorescences* about 4–6 cm. long, ♂ spicate, ♀ capitulate, with a central spicate axis ♂ and apparently past bloom when the ♀ flowers enter anthesis. *Perianth* ♂ delicate on a pedicel about 3 mm. long; petals ligulate,

hyaline, about 3 mm. long and wide; lobes (sepals) triangular-lanceolate or elliptic-lanceolate, about 3 mm. long and 1.5 mm. wide; androecium of about 15 stamens, the filament 4 mm. long, the anthers about 0.75 mm. long; glands usually 3, irregularly arranged, alternating with or opposing the lobes. *Perianth* ♀ very delicate, hyaline in texture; petals none; lobes 5 apparently fairly even, ligulate to sublinear, entire, with a thin thickened green midrib, about 7 mm. × 1 mm., becoming connate at base into the cup of the perianth, this sometimes bearing processes between the lobes; disc very thin, inconspicuous; ovary about 4–5 mm. large, somewhat ovoid-truncate, the endocarp slightly carinate at the upper end of the keel; styles 3, free, about 2 mm. long, each twice cleft, softly villous; seed about 3 mm. × 2 mm., ovoid, pointed at the upper end, the aril smooth, brownish with blackish and whitish irregular mottlings more numerous on the dorsal than on the ventral face, the caruncle apical, grayish, arrow-shaped, cleft in front.

HOLOTYPE: *V. L. Cory s.n.*, Padre Island, 26 mi. south of Port Aransas, November 1940 (Arnold Arbor.).

This is one of the peculiar endemics of Southwestern Texas. I am unable to state at this time which are its nearest affinities. Although it suggests *C. capitatus* Michx. in the details of its perianths it has a seed unlike any known to me in the species of the *C. capitatus* affinity. It appears likely that the 3 glands of the ♂ perianth are abortive stamens, their vasculation bearing to that of the subtending petal or lobe a relationship similar to that between an enation and the laminar body (petal or leaf) from which it arises. This disposition suggests that the ♂ perianth is undergoing regression, the outer stamens and their immediate appendages tending to fuse more or less irregularly and to depart from the normal quinary actinomorphic pattern of symmetry. A like process of degeneration is evident in the staminate flower of *C. parksii*, being manifested in this flower by the total elimination of the petals and by the more or less variable number of the lobes. The irregularly 6–8-lobed ♀ perianth of the species in the affinity of *C. capitatus* is probably a highly derivative structure arising by the fusion and subsequent reduction of the two normal perianth whorls (calyx and corolla); its peculiarities are strongly reminiscent of the ♀ perianth of *Julocroton*.

Croton albinoides (Ferg.) Croizat, comb. nov. *Croton engelmanni* var. *albinoides* Ferguson, Mo. Bot. Gard. Rept. **12**: 55. 1901.

Ferguson's *C. engelmanni* is invalid under the International Rules of Nomenclature, and must be replaced by *C. lindheimeri* (Engelm. & A. Gray) Wood, based upon *Pilinophytum lindheimeri*. Ferguson credits *C. lindheimeri* in his synonymy to Wood, Class Book of Botany, 631. 1865. This is an oversight; *C. lindheimeri* was used by Wood four years earlier in Class-Book Bot., 631. 1861.

Thanks to the friendly interest of Prof. J. M. Greenman I have had the privilege of inspecting the classic material now preserved in the herbarium of the Missouri Botanical Garden upon which Ferguson established his

understanding of *C. capitatus*, *C. engelmanni*, and *C. engelmanni* var. *albinoides*. Many of these specimens are duplicated in the Gray Herbarium, where I have also seen them. I believe that Ferguson is correct in treating *C. lindheimeri* as a species distinct from *C. capitatus*. These two entities differ both in intangibles and in characters and can be recognized without much difficulty by anyone who has learned how to identify them. The fact that it is often difficult, occasionally impossible, to determine *imperfect* specimens may not be assumed as a justification for treating these two species as one.

I find myself in disagreement with Ferguson, however, when he attaches his var. *albinoides* to *C. lindheimeri*, and this for the very cogent reason that the alleged variety differs as much from *C. capitatus* as it does from *C. lindheimeri*. Ferguson at one time recognized this, too, for he penned a note upon the sheet of *Heller 1800* in the Gray Herbarium to the effect that "This is typical of a very broad leaf form from S.W. Texas that shares characters from *C. capitatus* and *C. engelmanni*. It might form a subvariety under either. Ferguson Dec. 1897." It stands to reason that if a form is the connecting link between two others but cannot be brought under either on account of its strong individuality, either three separate species are involved in the complex or only one with two subordinate trinomials: both taxonomy and logic militate against two species with one variety being recognized in this case.

Ferguson's assumption that two species and one variety are involved rather than three species can easily be tested studying as a whole the group to which belong *C. capitatus* and *C. lindheimeri*. Leaving *C. albinoides* out of the reckoning, the species in this group are at least four—*C. capitatus* Michx., *C. lindheimeri* Wood, *C. muelleri* Coult., and *C. elliottii* Chapm. Of these four entities *C. elliottii* is the one that stands out most sharply despite the fact that it occasionally resembles depauperate specimens of *C. capitatus*. The affinities of *C. elliottii* lie in the immediate vicinity of *C. tenuilobus* S. Wats. (Mexico), *C. pedicellatus* H.B.K. (Colombia, Ecuador, Peru), *C. andinus* Muell.-Arg. (Bolivia, Argentina), and their allies in Paraguay and Brazil. Accordingly it would seem probable that the *C. capitatus* group has had its geographic and phylogenetic cradle in a geologic region now represented by southwestern Texas and that its affinities are to be sought with an Andine, i.e., prevailingly alpine element of tropical American flora. This, as we have already seen, is not the case with the group of *C. punctatus*, which is essentially Carribean and appears to have speciated from ancestral forms living along the strands of seas and other bodies of water. The fact that, as Ferguson notes, *C. albinoides* shares its range with *C. lindheimeri* but does not intergrade with it is characteristic of an entity that is already individualized and stable. Considering that Pleistocenic segregates of the

Euphorbiaceae in Argentina and in Europe are less stable in their morphology than *C. albinoides* (witness the polymorphism of the *Euphorbia portulacoides* and *Euphorbia esula* groups), there is ample justification on general grounds for interpreting this *Croton* as better than an occasional variety.

It is commonly understood that the seed furnishes the best characters of taxonomic and systematic determination in the Euphorbiaceae. Within certain limits I believe this to be true, especially in *Croton*. Whenever seeds are compared, however, a sharp distinction should be made between the aril and the testa, which recently (in Darwiniana **5**: 449. 1941) I have had reason to emphasize. The aril and its appendage, the caruncle, are not laid down and fully formed until the seed is ripe. The thickness and color of the aril, especially, vary during the evolution of the seed to maturity; unripe seeds have thin arils through which the testa is apt to show, but ripe seed may be so thickly coated by the aril so as not to show the testa at all. It also happens that plants grown under the best conditions may have slightly larger seeds than depauperate specimens; the aril itself may be thicker in response to edaphic conditions, it being likely that the more xerophilous members of an aggregate have thicker arils. I have unfortunately failed to secure fully ripe seed of *C. muelleri* but I have seen good seeds of *C. capitatus*, *C. lindheimeri*, and *C. albinoides*. Laid side by side the seeds of these four entities form a perfect series, as follows. (1) *C. capitatus:* seed lenticular, 5 mm. long and 5 mm. broad; aril thick, testa smoothish covered by the aril throughout. (2) *C. lindheimeri:* seed sublenticular, 4.5 mm. long and about 4 mm. broad; aril not very thick, testa finely rugulose-lacunose showing through the aril. (3) *C. albinoides:* seed ellipsoid, 4 mm. long and about 3 mm. broad; aril not very thick, testa rugulose-lacunose showing through the aril. (4) *C. muelleri:* seed nearly ripe and probably not materially different from a ripe seed, ellipsoid, 3 mm. long and 2.5 mm. broad; aril not very thick, testa as in *C. albinoides*. These four seeds are illustrated in figure 1 a, b, c, d, which should be compared with Ferguson's illustrations of the seed of *C. capitatus* (op. cit., pl. 18, 5), *C. lindheimeri* (as *C. engelmanni*, op. cit., pl. 19, 6), and *C. muelleri* (op. cit., pl. 20, 3). Clearly, the characters of these seeds are such that there is as much reason to treat *C. muelleri* as a variety of *C. albinoides* as there is for reducing *C. albinoides* trinomially under *C. lindheimeri*. Each of these seeds differs from every other in about the same degree, and taken together they bespeak uniform speciation within a fundamental consanguinity. In conclusion: four species are involved, not three species and one variety.

The evidence furnished by the seed that four species are actually involved is confirmed by other characters. The perianth of *C. lindheimeri* under a capsule about ready to dehisce is approximately 9 mm. long, while

it is fully one-third shorter in a comparable specimen of *C. albinoides* (fig. 1 e, f); the lobes are more or less irregular in *C. lindheimeri* but nearly or quite regular in *C. albinoides* (fig. 1 g, h). The indumentum of the flowers of *C. lindheimeri* has a marked yellowish or orangish cast, while that of

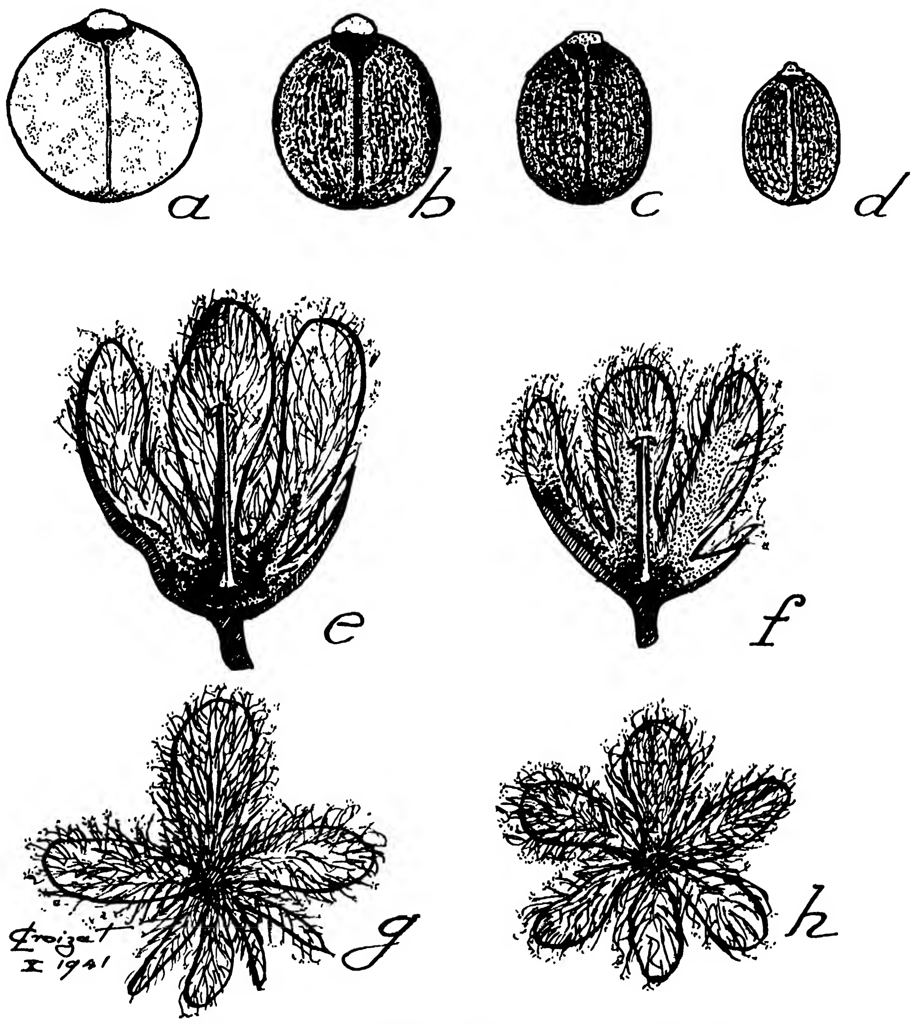


FIG. 1. Seed of (a) *Croton capitatus* Michx.; (b) *C. lindheimeri* Wood; (c) *C. albinoides* Croiz.; (d) *C. muelleri* Coult. A ♀ perianth in fruit of: (e) *C. lindheimeri*; (f) *C. albinoides* (sectioned to show details of the interior). A ♀ perianth in flower of: (g) *C. lindheimeri*; (h) *C. albinoides*. All drawings approximately to scale.

C. albinoides is whitish or grayish; Ferguson, as a matter of fact, uses the color to key *C. lindheimeri* out of *C. albinoides* (op. cit., 37) as follows: "Woolly tomentum of the calyx yellowish. *C. Engelmanni.* Appressed tomen-

tum white. *C. Engelmanni albinoides.*" It is true, moreover, that the tomentum is fluffier in *C. lindheimeri* than in *C. albinoides*, the difference in this respect being readily noticeable both in living and pressed specimens. Ferguson, lastly, establishes a definite separation between *C. lindheimeri* and *C. albinoides* when he states (op. cit., 55–56) that the latter grows with the former "but intermediate forms have not been observed." The range of *C. albinoides* is very pertinently indicated by him as "Southwest Texas and northward," thus localizing the species in southwestern Texas, whence it has radiated northward.

The following collections may be recorded for *C. albinoides*, (1) *Chandler 7075*, Rio Hondo, Cameron Co., Texas, Sept. 1913; (2) *Cory 17059*, 7 miles north of Sarita, Kenedy Co., Texas, Oct. 1935; (3) *Runyon 1985*, Boca Chica, Cameron Co., Texas, Sept. 1938; (4) *Cory s.n.*, Padre Island, 26 miles south of Port Aransas, Kleberg Co., Texas, Nov. 1940 (seeds about 5 mm. long, 4 mm. broad).

Pax & Hoffmann (in Natürl. Pflanzenf. **19**(c): 87. 1931) put *C. capitatus* Michx., *C. elliottii* Chapm., and *C. berlandieri* Muell.-Arg. under *Croton* subg. *Heptallon* (Raf.) Muell.-Arg. 1865. This listing contains several errors. Overlooking the lesser ones, such as the crediting of *C. berlandieri* to Mueller Argoviensis rather than to Torrey and the crediting of the subgenus to Mueller rather than to Pax himself, I should remark that, if published in 1865 or at any time thereafter, *Croton* subg. *Heptallon* is invalidated by *Croton* subg. *Pilinophytum* (Kl.) A. Gray (Man. Bot., ed. 2, xi, 391. 1856). A discussion of the nomenclature of this group in *the sectional rank* is contributed by Sprague (Kew Bull. **1929**: 82), cited by Pax & Hoffmann.

Croton subjucundus Croizat, sp. nov. Apparently a small shrub. *Innovations* at first thinly gray-tomentulose, very soon glabrous. *Leaves* 3–5 cm. long, 0.5–1.5 cm. broad, rather thin, drying a dark olive-green on both faces, glabrescent, lanceolate, bluntly rounded and mucronate at the tip, cuneate to round-cuneate at the base, with about 9 pairs of primaries, the basal sharply ascending, the next ones above ascending to broadly ascending, margins of the blade entire with few sessile or subsessile glands; petiole very slender, 1–2 cm. long, with a pair of glandular-setaceous stipules at the tip near the insertion of the blade and a tuft of glandular-setaceous stipules on either side at the base. *Inflorescence* a simple bisexual axis, the ♂ part seen only immature, apparently short. *Perianth* ♂ immature, not dissected. *Perianth* ♀ when fully spread out not over 5 mm. broad, the triangular-acuminate lobes not over 2 mm. long, bearing along their entire margin sessile or subsessile glands like those marginal to the leaf; petals as glands, altogether matching those at the apex of the petiole, apparently arising from the thin disc, this of 5 more or less connected glands, each gland opposing a lobe; ovary tomentellous to glabrate, grayish, smooth, globose-ovoid, about 2 mm. long and broad; styles 3, free, each 2–2.5 mm. long, dissected more or less irregularly, usually cleft into three main strips, the 2 lateral subfiliform,

the median one larger and once again dissected into 2 or 3 branches: seed ellipsoid, about 3.5 mm. × 3 mm., 2–3 times shallowly costate, the aril whitish, unevenly spread, filling the grooves and thickly covering the sides of the seed which appear whitish, elsewhere allowing the light-brownish testa to be seen.

HOLOTYPE: *Drouet & Richards 3923*, Navajoa, Sonora, Mexico, Dec. 1939 (Arnold Arbor.).

The holotype was distributed as *C. sonorae*, which is a patent misdetermination. *Croton subjucundus* is allied with *C. jucundus* Brandg. and *C. soliman* Schlect. & Cham., these two species being perhaps too close. I have carefully compared a good specimen of *C. jucundus*, which ranges nearest to *C. subjucundus* in Sonora, with the holotype of my species. *Croton jucundus*, as exemplified by *E. Palmer 1489*, has a ♀ perianth 10 mm. broad, with lobes about 5 mm. long, beset by numerous manifestly pedicelled glands, whereas *C. subjucundus* has a perianth barely 5 mm. broad, with lobes about 2 mm. long, bearing few short pedicelled or sessile glands. The styles of *C. jucundus* are at least three times as long as those of *C. subjucundus*, and its leaves bear at the margin many more long-pedicelled glands. It appears likely that the range of *C. subjucundus* is definitely more northern than those of its allies.

MANIHOT MILL.

Manihot walkerae Croizat, sp. nov. *Manihot carthagenensis*, Muell.-Arg. in DC. Prodr. **15**(2): 1073. 1866, quoad pl. Torrey, non Jacq. *Janipha loeflingii* Torrey in Emory Rept. U. S. Mex. Boundary Surv. **2**: 199. 1859, non H.B.K.

A low more or less scandent shrub. *Innovations* herbaceous, glabrous throughout and finely papillose under the lens when dry. *Leaves* glabrous, greenish above, more or less markedly glaucescent beneath, very variable in shape and size but essentially palmatilobed and peltate, 5–14 cm. long, 4–11 cm. broad; blade normally of 5 lobes around a subcentral peltate part, 1 lobe central, 2 lateral, these giving rise each to 1 lobe or lobule on the adaxial side; *central lobe* 3–7 cm. long, 1–3 cm. broad, ovate-lanceolate at the tip, scarcely mucronate or setulose, constricted 1–4 times more or less deeply at or below the middle, therefore repand pandurate, the lobulets arising by constriction patent, scarcely over 1.5 cm. long; *lateral main lobes* 2–6 cm. long, one occasionally missing, 0.5–3 cm. broad, constricted more or less like the median; *lateral secondary lobes* 2, arising from the lateral main ones, sometimes scarcely more conspicuous than lobulets by constriction, 0.5–3 cm. long, usually neither lobulate nor constricted; petiole slender, firm 3.5–8 cm. long, the stipules setaceous, inconspicuous. *Inflorescence:* the ♂ part a subspicate definite axis, the ♀ flowers practically free from the ♂ axis and basal to it, each flower usually borne upon its own pedicel, this arising directly or almost directly from the stem. *Perianth* ♂ definitely tubular and scarcely inflate at the base, borne on a pedicel not over 3 mm. long, the tube about 9–10 mm. long in anthesis, apparently inconspicuously streaked, the lobes 5, broadly lanceolate, 5–6 mm. long; stamens 10 (as seen; 8 described by Torrey) paired in the axils of lobulate glands, the filament about 8 mm. long, the anthers 2

mm. long. *Perianth* ♀ of lobes free to the base, only seen in a young fruit; lobes about 10 mm. long, 2.5–3 mm. broad, ligulate and rather bluntly acuminate, the disc manifest, bearing a few apparently imperfect stamens, their filaments usually persisting up to the fruit-stage; capsule on a pedicel 1.5–2.5 cm. long, more or less globose, about 12 mm. large, sparingly but manifestly verruculose and rugose, the 6 sutures (3 commissural, 3 dorsal) manifest; seed flattened, about 8 mm. long, 6 mm. broad, smooth, the aril ashen-gray with large blackish spots at the sides of the seed, especially on the dorsal face; caruncle large, 2 mm. long, about 4 mm. broad, yellowish, slightly cleft and 2-lipped in front.

HOLOTYPE: *E. J. Walker* (H. B. Parks) *s.n.*, along the lower Rio Grande, south of Mission, Hidalgo Co., 1940 (in herb. Arnold Arbor.).

The type material was collected by Mrs. E. J. Walker of La Joya and transmitted by Mr. Parks through Mr. Cory. The two sheets of *Schott's* collection at the Ringgold Barracks, 1853, seen by Torrey and identified by him as *Janipha loeflingii* and *Janipha loeflingii* var. *yuquilla* are now preserved in the herbarium of the New York Botanical Garden, bearing Britton's determination, *Manihot carthagenensis* Muell.-Arg. *Janipha loeflingii* is an invalid renaming of *M. carthagenensis,* and *J. yuquilla* is probably little better than a leaf-form of the same species. It appears that Mueller Argoviensis was misled by the erroneous determination of Torrey into bringing the Texan plant into the synonymy of the Colombian *Manihot* where it has remained ever since 1866. That the species are different is clear, the perianth, not to mention other less immediately evident characters, being short and inflated in *M. carthagenensis,* rather narrow and tubular in *M. walkerae.* The resemblance in the foliage is close, however: in certain specimens of *M. carthagenensis* or forms near it (for instance, *Seler 2814*, Guatemala, 1896) the leaf has very much the same aspect and outline of that of the holotype of *M. walkerae* and of Schott's collections. Pax (in Pflanzenr. 44 (iv. 147. ii): 81. 1910) does not list nor mention Texan material under *M. carthagenensis.* Coulter, on the contrary, follows the lead of Mueller (Contr. U. S. Nat. Herb. **2**(3): 400. 1894) and it is on Coulter's authority rather than on that of others that *M. carthagenensis* has erroneously been received in the flora of Texas. *Manihot carthagenensis* is a critical species which it should be desirable to typify as there is no lack of evidence that several species are confused under this binomial in tropical America as well as in Mexico.

In the notes and sketches attached to the Schott specimens Torrey states that the stamens vary from 8 to 10 and mentions that some of them are longer. Whether this is true or not I may not definitely say: I have found only 10 stamens without the appearance of a superimposed verticil in the few flowers which I have dissected. I have now a plant of *M. Walkerae* in cultivation in the hothouse and, should it blossom, it will be possible to verify the constancy of the stamens both in number and in length.

Manihot is a genus of great interest to students of carpellary structures. The vasculation of the epicarp arises mostly *from the columella*, and it streams to the epicarp through peculiar gaps between the so called carpels, this being accurately figured by Baillon (Etud. Gén. Euphorb., pl. xix, 15. 1858). There is, moreover, a very strong indication that abortive stamens may enter in the making of the epicarp of the Euphorbiaceae or, at least, may become intimately adnate with it, nuptial nectaries being found scattered upon the ovary of *Podadenia thwaitesii* Muell.-Arg. and, occasionally, of species of *Codiaeum* A. de Jussieu.

TETRACOCCUS ENGELMANN EX PARRY

Wheeler, I believe, has written the most recent study of *Tetracoccus*. In this review (Contr. Gray Herb. **127**: 50–53. 1939) he eventually concludes that *Tetracoccus* and *Halliophytum* cannot be maintained as separate genera. Unfortunately, this conclusion has no effect, because Wheeler fails to follow it by the reductions and transfers which are required to make it taxonomically viable.

In the course of abundant bibliographical and nomenclatural remarks Wheeler affirms that *Bernardia(?) fasciculata* S. Wats., the basinym of *Halliophytum fasciculatum* Johnst., is a provisional name to be rejected under Art. 37 *ter* of the International Rules of Nomenclature. There is no reason for me to take up this affirmation and, even less, to comment upon Cook's more recent declarations on the same subject (in Jour. Wash. Acad. **31**: 51. 1941), because I have dealt with *nomina provisoria* on two previous occasions (Jour. Arnold Arb. **21**: 499. 1940; **22**: 137. 1941). Since, however, it is desirable that the already involved nomenclature of *Tetracoccus* should not be again disturbed by misinterpretations of the Rules, I will briefly deal with the concept of "validation" as this may apply to Johnston's use of *Bernardia(?) fasciculata* as basinym for *Halliophytum fasciculatum*.

The animadversions contributed by Wheeler in the cited review and his later discussion of the meaning and purposes of Art. 69 (Am. Midl. Nat. **21**: 529. 1939) indicate that his understanding of this Article is not altogether clear. Article 69 offers a solution to a fundamental nomenclatural difficulty which is the cleverest to be found in the whole body of the Rules. The author of this solution fully understood that the recognition of the validity of a combination based upon an illegitimate name would conflict with the basic principle (Art. 2) that an illegitimate name *must* be rejected. He realized, on the other hand, that the rejection of combinations already effected around illegitimate names would disturb the stability of nomenclature which is the foremost concern of the Rules (Art. 4). As a way out of this dilemma the author of Art. 69 devised the altogether elegant solution of treating combinations made around illegitimate names as *new names*. To elucidate, using

the example in Art. 69: *Talinum polyandrum* Hook., being illegitimate, could not be accepted as the source of *Calandrinia polyandra* (Hook.) Benth. *comb. nov.* The specific epithet *polyander* could be freely used, however, to propose *Calandrinia polyandra* Benth. *nom. nov,* naturally, so long as no other earlier homonym stood in the way of this binomial. In other words: the author of Art. 69 decided that the use of an illegitimate epithet in a new combination justifies merely a change in authorship, not the rejection of the binomial. Thus he automatically reconciled the stability of nomenclature (Art. 4) with the principle that an illegitimate name must be rejected (Art. 2). That this is a wise and able solution does not need to be emphasized in detail. Fully recognizing the value of the solution offered by the author of Art. 69 Rehder has proposed (Jour. Arnold Arb. **20**: 276. 1939) that this solution be definitely embodied in the text of the Article itself rather than be attached, as it were, to its end. I may add that it should be desirable to extend to trimonials (Art. 58) the principle that a combination based on an illegitimate name is a *new name*. Be this as it may, Johnston had a clear case for publishing *Halliophytum fasciculatum*. This binomial would be treated as a *new name* (Art. 69) in the event that *Bernardia(?) fasciculata* should prove to be illegitimate. It would be treated as a *new combination,* on the contrary, if *Bernardia(?) fasciculata* turned out to be a legitimate binomial. Thus, nothing more relevant can be involved in Wheeler's animadversions and discussions than the fact whether the correct citation is to be *Halliophytum fasciculatum* Johnst. or *H. fasciculatum* (S. Wats.) Johnston. Since there is not the slightest doubt about *Bernardia(?) fasciculata* being a legitimate name and not a *nomen provisorium*, the correct citation is *Halliophytum fasciculatum* (S. Wats.) Johnst., and *Tetracoccus fasciculatus* (S. Wats.) Croizat.

It is true that *Halliophytum* is not separated from *Tetracoccus* by sharp generic distinctions. Very few genera of the Euphorbiaceae, as a matter of fact, are separated from their nearest allies by conventionally "strong" characters, and nothing could be more easily done than to "prove" that *Mallotus* and *Macaranga*, *Andrachne* and *Phyllanthus*, *Chamaesyce* and *Euphorbia*, *Sapium* and *Stillingia* are not all "good" genera. Such a "proof," however, would lead taxonomy nowhere, for it would merely lead to the conclusion that since every genus is another genus and every family merges with some other it is useless to do any taxonomic work at all.

Tetracoccus and *Halliophytum* differ in the details of their foliage, in certain characters of their capsules, and, which is more important, in the arrangement of their inflorescences. If these two genera had been in use for a century they would be kept separate today on grounds of tradition and convenience in the same manner as Hooker maintains *Glochidion* (Fl. Brit. Ind. **5**: 306. 1887) and Prain upholds *Excoecaria* (Fl. Trop. Afr. **6**(1): 1018.

1913). Likewise, if these two genera had each numerous species or occurred in separate regions they would be recognized as are *Andrachne* and *Phyllanthus, Mercurialis* and *Claoxylon*. A very strong case indeed could be made for maintaining *Halliophytum* if the discovery of *Tetracoccus ilicifolius* Cov. & Gilm. had not introduced a new element in our understanding of the range and morphology of this group. We know today what was not as yet known to Johnston when he published *Halliophytum;* the species in this complex are few and fall into three rather than only two main groups. We know, moreover, that the systematic position of this entire aggregate is peculiar. *Tetracoccus ilicifolius* is reminiscent of *Toxicodendron globosum* (Gaertn.) Pax & Hoffm., a monotype of South Africa, of which it practically has the ♂ inflorescence. *Tetracoccus capensis*, on the other hand, suggests the characters of *Securinega virgata* (Poir.) Maire, endemic to Portugal and allied with *S. leucopyrus* Muell.-Arg., from India, and *S. acicularis* Croiz., from Central China. It might be inferred from these affinities that the surviving American and Mexican oligotypes under *Tetracoccus* and *Halliophytum* are relics from an aggregate which was originally far richer in diversified forms than it is at present, and was closely related with forms which today we must accept as unrelated because the connecting links are extinct. Clearly, *Tetracoccus* and *Halliophytum* stand at the threshold of a full generic segregation, and the latter of these two names must be regarded as a subgenus of the other. That *Tetracoccus* is not congeneric with *Securinega* will be evident to anybody who critically studies the two entities.

In agreement with the previous conclusions, I propose the following classification:

Tetracoccus Engelm. ex Parry in West. Am. Scient. **1**: 13. 1885; Pax & Hoffm. in Natürl. Pflanzenf. **19**(c): 74. 1931; Munz, Man. S. Calif. Bot. 282. 1935; McMinn, Man. Calif. Shrubs 252–253, figs. 286–287. 1939; Wheeler in Contr. Gray Herb. **127**: 50–53. 1939.

Bernardia (?) S. Wats. in Proc. Am. Acad. **18**: 153. 1883; Brandeg. in Zoë **4**: 405. 1894. Non Adans.

Securinega, I. M. Johnst. Univ. Calif. Publ. Bot. **7**: 441–442. 1922; Jepson, Man. Fl. Plants. Calif. 593. 1925. Non A. L. de Juss.

Halliophytum I. M. Johnst. Contr. Gray Herb. n.s. **68**: 88. 1923; Munz, op. cit. 283; McMinn, op. cit. 249, fig. 282.

NOMENCLATURAL TYPE: *Tetracoccus dioicus* Parry.

A. **Eutetracoccus** Croizat, subg. nov.

NOMENCLATURAL TYPE of the genus; monotypic.

Inflorescence ♂: cymose racemes, sometimes leafy or bracteate-leafy. Inflorescence ♀: flowers mostly single, peduncles and pedicels elongate, persistent, ultimately woody; capsule hard, sublignescent. Leaves subentire, nearly alternate to alternate or fascicled in clusters of few.

B. **Halliophytum** (Johnst.) Croizat stat. nov.

NOMENCLATURAL TYPE: **Tetracoccus fasciculatus** (S. Wats.) Croiz. comb. nov. (*Bernardia*(?) *fasciculata* S. Wats. Proc. Am. Acad. **18**: 153.

1883; *Halliophytum fasciculatum* Johnst. Contr. Gray Herb. n.s. **68**: 88. 1923.

Two species in addition to the nomenclatural type, (a) *T. hallii* Brandeg. Zoë **5**: 229. 1906; (b) **T. capensis** (Johnst.) Croiz., comb. nov. (*Securinega capensis* Johnst. Univ. Calif. Publ. Bot. **7**: 441. 1922).

Inflorescence ♂: flowers single or few together borne as a rule on brachyblasts. Inflorescence ♀: flowers single or nearly single, peduncles and pedicels delicate or, at least, shorter and smaller than those of Subg. *Eutetracoccus;* capsule small, more or less elongate, firmly coriaceous rather than lignescent. Leaves small, entire, fascicled or congested, alternate.

C. **Tetracoccaster** Croizat, subg. nov.

NOMENCLATURAL TYPE: *Tetracoccus ilicifolius* Cov. & Gilm. in Jour. Wash. Acad. Sc. **26**: 531. 1936. Monotypic.

Inflorescence ♂: racemose or cymose, elongate, the flowers reduced and subglomerulate along a main axis. Inflorescence ♀: like that of *Eutetracoccus.* Leaves alternate, subopposite, opposite or verticillate manifestly serrate.

In closing this brief review of *Tetracoccus* I must thank Mr. M. F. Gilman, co-author of *T. ilicifolius,* for his truly inexhaustible patience in supplying me with pressed specimens, live cuttings, and complete flowering and fruiting material of this remarkable species.

LATIN DIAGNOSES

Croton parksii Croiz., sp. nov. Fruticulus; foliis tomentosis, *C. capitatum* Mchx. in mentem vocantibus, plerumque ellipticis, 2.5–6 cm. longis, 1–2 cm. latis, petiolo ad 1 cm. longo. Inflorescentiis abbreviatis subspicatis vel racemosis, in ♀ capitulatis. Perianthio ♂: petalis nullis, lobis 6(–5), staminibus 10–15 intra discum glandulosum impositis, pedicello 2 mm. longo. Perianthio ♀: petalis nullis, lobis triangulari-ellipticis, disco tenui, capsula muricata, tomentosa, ca. 10 mm. magna: semine 7 mm. longo 6 mm. crasso, caruncula valida apicem seminis totius obtegente.

Croton coryi Croiz., sp. nov. Fruticulus, apicibus grosse hispido-tomentosis. Foliis hispido-tomentosis 3.5–5 cm. longis, 1.5–2 cm. latis, ellipticis vel elliptico-lanceolatis; petiolo 1.5–2.5 cm. longo. Inflorescentiis subspicatis vel capitulatis. Flore ♂ delicato, hyalino, petalis sepalisque 5, glandulis ca. 3, staminibus ca. 15. Flore ♀: Perianthio hyalino, lobis 5, ligulatis ad 7 mm. longis, petalis nullis, disco obscuro, ovario 4–5 mm. magno, stylis 3 liberis ca. 2 mm. longis; semine acutato, brunneo-griseo, levi, caruncula apicali sagittata.

Croton subjucundus Croiz., sp. nov. Fruticulus, apicibus griseo-tomentellis dein glabratis. Foliis lanceolatis 3–5 cm. longis, 0.5–1.5 cm. latis, apice abrupte rotundato-mucronatis margine glandulis sessilibus vel subsessilibus paucis obsito, venis ca. 9-jugis, petiolo gracili, apice glandulis binis, basi glandulis plurimis totis setaceo-glandulosis obsito. Flore ♂ haud viso. Flore ♀: perianthio 5 mm. lato, lobis triangularibus ad 2 mm. longis, petalis glandulosis; ovario ca. 2 mm. magno, stylis 3 liberis 2–2.5 mm. longis, in lacinias dissectis: semine ellipsoideo, grosse costato, arillo tenuiore, albicante.

Manihot walkerae Croiz., sp. nov. Frutex plus minusve scandens, innovationibus herbaceis. Foliis 5–14 cm. longis, 4–11 cm. latis, mire ludentibus,

pro more palmatim 5-lobis, peltatis, lobis constrictis vel panduratis. Inflorescentia racemosa, basi ♀. Perianthio ♂: tubulari apice 5-lobato, haud inflato, ca. 10 mm. longo, 12–16 mm. lato, staminibus ca. 10, binis in axilla glandulae retusae. Perianthio ♀: ad basim partito, 5-lobo, lobis ca. 10 mm. longis sub fructu immaturo: disco staminibus paucis onusto: capsula verruculosa, 12 mm. magna, globosa: semine lenticulari 8 mm. longo, 6 mm. lato, arillo cinereo, maculis nigris praesertim ad latera dorsumque notato.

Eutetracoccus Croiz., subg. nov. Inflorescentia ♂: racemis cymosis, interdum foliosis vel bracteato-foliosis. Inflorescentia ♀: floribus plerumque singulis, pedunculis pedicellis elongatis, lignescentibus, persistentibus; capsula dura, lignescente. Foliis subintegris, subalternis vel alternis vel paucis fasciculatis.

Tetracoccaster Croiz., subg. nov. Inflorescentia ♂: racemosa, vel cymosa, elongata, floribus diminutis, secum rachidem subglomeratis. Inflorescentia ♀: cum illa *Eutetracocci* congruente. Foliis denticulatis, ilicinis.

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NOTE: This paper had been typewritten and sent to the editor, when I received from Mr. C. T. White, of the Botanic Gardens of Brisbane, Queensland, Australia, seeds of *Petalostigma glabrescens*, which I had requested a long time ago to effect a comparison with the seeds of *Tetracoccus*.

Both the seed of *Petalostigma* and *Tetracoccus* have a glossy vitreous aril, flesh-colored in the former, nearly brick-colored in the latter, which is immediately recognized by those who have once seen it. An evolute caruncle also occurs in both genera, the micropyle being reached through a more or less tubular opening in the caruncular tissues or folds. The raphe is external, that is, it runs through the aril, close to the testa but not within the testa, as it does in other Euphorbiaceae, nor under it. The chalaza is fairly well marked or well marked, the chalazal vascular plug being manifest. The differences between the seed of *Petalostigma* and *Tetracoccus*, exemplified, respectively, by *P. glabrescens* and *T. ilicifolius* are on the whole of little moment. In the former, which is about 8–9 mm. long, the micropylar end of the testa is produced into a thin point, the radicle being practically vertical in relation to the longer axis of the seed; in the latter, 4–5 mm. long, the radicle is slightly deflexed, and a considerable thinning out of the testa takes place under the caruncle. The endosperm is about of the same amount, texture and color in the two species.

The seed of *Securinega virosa* (*Flueggea virosa*) departs from the seed of *T. ilicifolius* in the lack of the caruncle and in minor structural details, but agrees in other respects, such as in the presence of an endosperm. A logical interpretation of these three seeds is that the ancestral form is that of *Petalostigma*, the seed of *Tetracoccus* being intermediate between that of *Petalostigma* and *Securinega*. In many respects, *Petalostigma* is strongly

reminiscent of *Beyeria* and *Ricinocarpus*, forming with them a group which it is possible to tie in phylogeny with the Sterculiaceae of the *Keraudrenia*—*Seringia* affinity in a very conclusive manner.

Since these Sterculiaceae are also very near the probable ancestors of *Croton* it is possible to visualize the following lines of descent: The ancestral sterculioid form, derived from the malvoid plexus by a definite fixation of the tendency towards apetaly and unisexuality first appearing in certain

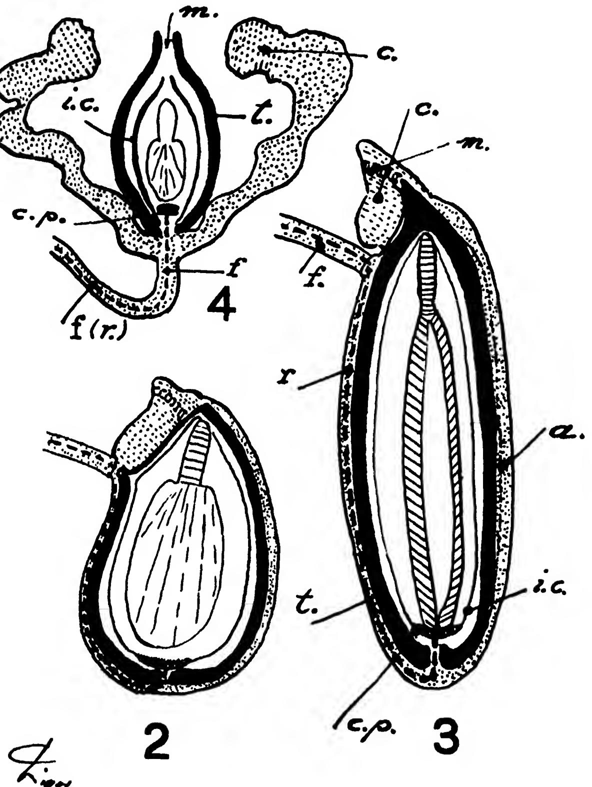

FIG. 2. Section through seed of *Tetracoccus ilicifolius*. FIG. 3. Section through seed of *Petalostigma glabrescens*. FIG. 4. Diagram of ancestral ovule. For explanation see text.

Malvaceae, yields two main streams of evolution, one that moves towards the *Croton* aggregate, the other towards the *Phyllanthus* group. At the point of common origin of these two streams are found *Ricinocarpus, Beyeria, Longetia, Toxicodendron* and *Petalostigma*. Moving further in the direction of *Phyllanthus* we see *Putranijva* and *Drypetes* appear together with *Tetracoccus*. Next comes *Securinega* and last *Phyllanthus*, the largest and the most polymorphous of all the genera mentioned. These conclusions substantially

agree with those advanced in the previous article to the effect that *Tetracoccus* is a relic from a group that is less advanced than *Phyllanthus* but as yet not too far from, and possibly earlier than *Securinega.*

Figures 2–4 illustrate a section through the seed of *Tetracoccus ilicifolius* (fig. 2) and *Petalostigma glabrescens* (fig. 3). The fibro-vascular system of the funiculus (f) furnishes the raphe (r), which runs within the aril (a) and enters the chalaza at the base of the seed passing through an evident opening in the testa. The chalazal vascular plug (c.p.) is marked, its branches running, as it seems, throughout the inner coat (i.c.). The micropyle is reached by the micropylar opening (m.) penetrating the caruncle (c.). It is interesting to compare this disposition with that of the presumed ancestral orthotropus ovule (fig. 4), which shows the caruncle as a mere labial expansion of the aril closing upon the micropyle. The passage from the anatropous pattern of *Petalostigma* and *Tetracoccus* to the presumed orthotropus disposition of the ancestor is a matter of but slight changes, involving in the main the fusion of the funiculus with the opposite wall of the aril as shown in figure 4—the funiculus thus becoming in part the raphe (r. [r.])—and the molding of the aril upon the testa to form a single, close structure. Thus the basic coats of the seed, aril, testa and inner coat are suggested as enations from the torus ending the funiculus.

This diagram is fundamental for the seed-structures of all the Euphorbiaceae, despite the fact that very great differences are found in their seeds, due to various degrees of connation and adnation between the structural components of the seed-wall, the evolution or suppression of these components and the location of the seed-reserves within the testa or, apparently, in the tissues of the aril. The obturator, as it is well known (Schweiger, Flora **94**: 379. 1905), does not belong to the seed proper but to the placenta and, perhaps, to the margins of the carpels. The caruncle, however, belongs to the seed-coat (Schweiger, loc. cit.), as shown in my diagram.

It will be noticed that the structural premises of the seed of *Petalostigma* and *Tetracoccus* are practically the same, the anatomic unity of the organs in these seeds showing that the peculiar aspect of the aril of these two genera has considerable significance as an index of consanguinity.

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ASA GRAY'S EARLIEST BOTANICAL PUBLICATIONS (1833–1836)

H. W. RICKETT AND C. L. GILLY

"NORTH AMERICAN GRAMINEAE AND CYPERACEAE"

Asa Gray's first publication was entitled "A catalogue of the indigenous Flowering and Filicoid plants growing within 20 miles of Bridgewater, (Oneida Co) N. Y." It appeared in the 46th Annual Report of the Regents of the University of the State of New York (Albany, 1833); the obscurity of this publication may account for the absence of the title from the well known bibliography of Gray's work by Watson and Goodale (Am. Jour. Sci. **136** (Appendix): 1–42. 1888). The catalogue listed 785 species and 5 varieties in 352 genera. There are no descriptive notes, or introductory statements; the species are classified in the De Candollean system as modified by Lindley. Gray thought he had new species of *Scirpus, Erysimum,* and *Ophioglossum,* but did not name or describe them. The catalogue is noteworthy for its Cyperaceae; the 60 species of *Carex* and 11 of *Scirpus* attest his early interest in this group.

Gray's second publication in botany[1] was a century of exsiccatae accompanied by printed descriptions and synonymy, a title page, dedication, introduction, and index, entitled *North American Gramineae and Cyperaceae, Part I.* This work, issued early in 1834,[2] was supported by subscription. The demand exceeded the supply; later in the year he speaks[3] of another collecting trip to secure specimens for additional sets. That all of the examples of a given number were perhaps not collected at the same time may explain the fact that some numbers are represented by a mixture of species (see below); since some were new species, the importance of such facts is evident.

The sets, at least some of them, seem to have been issued unbound and probably unmounted. In June Gray wrote to Torrey:[4] "It will be best by all means not to trouble yourself in preparing the 2 copies of Gram. & Cyp. left with you. I will have them, with several others bound up early in the fall." The labels were printed in two columns on large sheets of paper, with

[1] Between his first two botanical works he wrote, with J. B. Crawe as senior author, "A sketch of the mineralogy of a portion of Jefferson and St. Lawrence Counties (N. Y.)" (Am. Jour. Sci. 25: 346–350. 1834).

[2] The date is established by a letter to Torrey, dated 8 May 1834, now in the library of the New York Botanical Garden. This letter and others to which reference is made have been published in *Letters of Asa Gray,* edited by Jane Loring Gray (1893).

[3] Letter to Torrey, 9 June 1834.

[4] This is from the letter of 9 June 1834 referred to above. These sentences are written in the margin and are not included in the published version of the letters.

guide lines to show where they should be cut. A bound copy of Part I is preserved in the library of the New York Botanical Garden; several additional specimens with identical labels are scattered through the herbarium. A similar dispersed set is in the Gray Herbarium, and a bound copy is in the herbarium of the Philadelphia Academy of Natural Sciences. Other bound or dispersed sets are undoubtedly in existence. The paper used for mounting the specimens in the New York copy of Part I differs from that upon which the labels and introductory matter are printed and appears to be the same as that used in mounting contemporary specimens of the Torrey Herbarium.

The second century (*North American Gramineae and Cyperaceae, Part II*) was issued in 1835[5] apparently in much the same form as Part I. A complete bound set of this issue of exsiccatae is preserved in the herbarium of the Philadelphia Academy of Natural Sciences.[6] The paper on which title page, index, and printed labels are printed appears to be of the same kind as that used in Part I. The printing of both was done by J. Post, Printer, 101 John Street, New York. A considerable number of separate sheets of this second century are in the herbarium of the New York Botanical Garden, and other sets are doubtless on file elsewhere. As additional evidence that these exsiccatae were issued as loose sets of specimens, and not as bound volumes, the mounting paper used in the Philadelphia copy of Part II differs from that of the New York copy of Part I, as well as from that on which title page, index, and labels were printed.

The collections were made largely in the pine barrens and on the coast of New Jersey and in the central and western parts of New York. A passage in Gray's autobiography[7] fixes the dates of his collections in New Jersey as 1833 and in New York as between 1828 and 1835.

The principal interest of these sets is that in them new species were described, new combinations made, and new names given. Some of these had been named by Torrey while preparing his (unpublished) Synoptical Flora; though their authorship is quite clearly indicated by Gray, they have been frequently wrongly ascribed to Gray, or sometimes to Torrey with erroneous citation of the place of publication. Some of these errors derive from the fact that certain authors have not regarded the *Gramineae and Cyperaceae* as a publication; many of the references to Trinius, when followed up, show that he had access to a set of this work and was one of the first to use the names there published (see, for instance, *Muhlenbergia sylvatica* below). The new species, combinations, and names (principally

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[5] Letter to W. J. Hooker of 4 April 1835.

[6] We are glad to acknowledge the courtesy of Dr. F. W. Pennell in making this volume available for our examination.

[7] Published in *Letters of Asa Gray*.

nomina nuda), with their present status, and some of the most widely quoted erroneous references are listed below.

VILFA SEROTINA Torrey in Gray, N. Am. Gram. & Cyp. **1**: 2. 1834 (based on *Agrostis serotina* Torrey. 1823), nomen superfluum. Hitchcock (Man. Gr. U. S. 895) includes under *Muhlenbergia uniflora* (Muhl.) Fernald: "*Vilfa serotina* Trin., Gram. Icon. 3: pl. 251. 1830. . . . '*Agrostis serotina* Nutt. MS.' " This was apparently an independent and earlier publication of the same name for the same species, based on a different type. Both *V. serotina* Torr. and *V. serotina* Trin. must be regarded as new names rather than as new combinations since both *A. serotina* Torrey and *A. serotina* Nutt. are later homonyms of *A. serotina* Lam. (1767), a distinct species. The Index Kewensis wrongly ascribes *V. serotina* to "Torr. ex Trin. Sp. Gram. iii, t. 251."

VILFA VAGINIFLORA Torrey in Gray, N. Am. Gram. & Cyp. **1**: 3. 1834 (nomen novum for *Agrostis virginica* Muhl. and other authors. Not *A. virginica* L. 1753), = *Sporobolus vaginiflorus* (Torr.) Wood, Class Book 775. 1861; so cited by Hitchcock (Man. Gr. U. S. 961). In his *Flora of New York* (**2**: 438. 1843) Torrey had used the spelling *vaginaeflora,* which was adopted by Wood, and by Britton and Brown (Ill. Fl. ed. 2, **1**: 194); the latter ascribed the new combination to "Torr.; Wood Classbook 775. 1861." The Index Kewensis has "*V. vaginiflora* Torr. ex. Trin. in Mém. Acad. Pétersb. Ser. VI. Sc. Nat. v. ii. (1840) 56."

VILFA LONGIFOLIA (Torrey) Torrey in Gray, N. Am. Gram. & Cyp. **1**: 4. 1834 (based on *Agrostis longifolia* Torrey, Fl. U. S. **1**: 90. 1823). Hitchcock (Man. Gr. U. S. 958), under *Sporobolus asper* (Michx.) Kunth, attributes this name to "Torr.; Trin., Mém. Acad. St. Pétersb. VI. Sci. Nat. 4: 107. 1840." In the Index Kewensis, the place of publication is similarly stated.

MUHLENBERGIA SYLVATICA (Torrey) Torrey in Gray, N. Am. Gram. & Cyp. **1**: 13. 1834 (based on *Agrostis sylvatica* Torrey, Fl. U. S. **1**: 87. 1823). This is recognized as a valid name by Hitchcock (Man. Gr. U. S. 894) but with the erroneous citation: "Torr.; Trin. Mém. Acad. St. Pétersb. VI. Sci. Nat. 4: 292. 1841." Trinius has "*M. sylvatica* Torr.! Synop. Flor. Ined." Britton and Brown (Ill. Fl. ed. 2, **1**: 186), in synonymy under *M. umbrosa,* give the place of publication as "Cat. Pl. N. Y. State 188. 1840."

AGROSTIS MICHAUXII var. LAXIFLORA (Michx.) Gray, N. Am. Gram. & Cyp. **1**: 17. 1834 (based on *Trichodium laxiflorum* Michx. 1803). This combination is not listed by Hitchcock in the Manual; *Trichodium laxiflorum* Michx., however, is included in the synonymy of *Agrostis hiemalis* (Walt.) BSP. (Man. Gr. U. S. 781).

CALAMAGROSTIS COARCTATA (Torrey) Torrey in Gray, N. Am. Gram. & Cyp. **1**: 19. 1834 (based on *Arundo coarctata* Torrey. 1823). Hitchcock (Man. Gr. U. S. 820), under *Calamagrostis cinnoides* (Muhl.) Barton, has "*Calamagrostis coarctata* Torr.; Hook., Fl. Bor. Amer. 2: 240. 1840. Presumably based on *Arundo coarctata* Torr. Published as new in Torr., Fl. N. Y. 2: 444. pl. 151. 1843. Based on *A. coarctata* Torr." In both the Hooker

and Torrey publications cited by Hitchcock, the combination is correctly cited. The Index Kewensis ascribes *C. coarctata* to "Torr. Fl. N. York, ii. 444."

CALAMAGROSTIS EXPANSA Gray, N. Am. Gram. & Cyp. **1**: 20. 1834. Hitchcock (Man. Gr. U. S. 820) accepts and correctly cites this species. In the Index Kewensis, the citation is "A. Gray, ex Torr. Fl. N. Y. ii. 445. t. 152 [1843]." Britton and Brown (Ill. Fl. ed. 2, **1**: 210) refer the species to "A. Gray; Torr. Fl. U. S. **2**: 445. 1843."

PANICUM XANTHOPHYSUM Gray, N. Am. Gram. & Cyp. **1**: 28. 1834. Hitchcock (Man. Gr. U. S. 916) regards this as a good species and cites it correctly. The Index Kewensis has "A. Gray, Man. Bot. N. U. S. St. ed. 1. 613. [1848.]" Britton and Brown (Ill. Fl. ed. 2, **1**: 158) refer it to "Ann. Lyc. N. Y. **3**: 233. 1835."

PANICUM NITIDUM var. CRASSIFOLIUM Gray, N. Am. Gram. & Cyp. **1**: 30. 1834, nomen nudum. Hitchcock (Man. Gr. U. S. 913) under *Panicum sphaerocarpon* Ell., ascribes this variety to "A. Gray; Doell, in Mart., Fl. Bras. 2: 247. 1877." Doell (l.c.) attributed the variety to Asa Gray, provided it with a brief description, but said that it was a different species. Whether this can be said to "validate" Gray's nomen nudum is debatable.

CYPERUS MARISCOIDES var. SETIFOLIUS Torrey in Gray, N. Am. Gram. & Cyp. **1**: 75. 1834, nomen nudum.[8] This varietal name, though cited by Torrey in synonymy under *C. grayii* (Fl. N. Y. **2**: 342. 1843), has apparently never been provided with a description.

ERIOPHORUM POLYSTACHION var. TENELLUM (Nutt.) Torrey in Gray, N. Am. Gram. & Cyp. **1**: 91. 1834 (based on *E. tenellum* Nutt. 1818). Fernald (Rhodora **7**: 87. 1905) gave the correct citation for this combination, but erroneously attributed it to Gray.

PANICUM NITIDUM var. VILLOSUM Gray, Gram. & Cyp. **2**: 111. 1835. This should probably be regarded as a new name. In the synonymy of this number Gray cites, "P. pubescens. *Lam'k. Enc. Meth.* IV. *p.* 748? *Muhl. Gram.* p. 116?"

PANICUM NITIDUM var. ANGUSTIFOLIUM (Ell.) Gray, Gram. & Cyp. **2**: 112. 1835 (based on *P. angustifolium* Ell. 1816). This is obviously based on *Panicum angustifolium* Ell. although Gray made a double error in the citation of the name-bringing synonym: "*P. angustifolium* Trin. Gram. Panic. p. 223." On this page only two specific names appear: *P. lancearium* Trin. (which is the second synonym cited by Gray for the specimen now under discussion) and *P. clandestinum* L. It would seem quite logical to assume that the "223" of Gray's citation is merely a typographical error for 234, on which page *P. angustifolium* Ell. is mentioned; and that in accrediting the species *P. angustifolium* to Trinius he was merely following the common

[8] Those who still recognize the distinctions made in the "American Rules" of 1907 will regard this and several other names here listed as hyponyms rather than nomina nuda.

practice of that day, of referring to a publication at hand which contained a description of a species originally published by another author. Except for the glabrous leaf sheaths, the specimen of no. 112 in the Philadelphia copy of Part II matches the description of *P. angustifolium* given by Trinius.

URACHNE CANADENSIS (Poir.) Torrey in Gray, Gram. & Cyp. **2**: 114. 1835 (based on *Stipa canadensis* Poir. 1806). This specific name, included in synonymy under *Oryzopsis canadensis* (Poir.) Torr. by Hitchcock (Man. Gr. U. S. 897), is accredited by him to "Tor. & Gray;" also, although he correctly cites the place of publication, the date is given as "1836." In the Index Kewensis the citation for this name is "Torr. & Gray, ex Trin. & Rupr. l.c. v(1842) 17 [Sp. Gram. Stip.]." Examination of this publication reveals that Trinius and Ruprecht attributed the name to "Torr. & Gray! Gram. et Cyp. exsicc. (1836) n. 14," and cited it only as a synonym.

TRITICUM REPENS var. GLAUCUM Gray, Gram. & Cyp. **2**: 128. 1835, nomen nudum. So far as we can determine the variety has never been described.

CAREX PENSYLVANICA var. MUHLENBERGII Gray, Gram. & Cyp. **2**: 163. 1835 (nomen novum for *C. varia* Muhl. ex Wahl. 1803). Mackenzie (N. Am. Fl. **18**: 194. 1935) cites this name in synonymy under *C. communis* as follows: "*Carex pennsylvanica* var. *Muhlenbergii* A. Gray (N. Am. Gram. *163*. 1835); Torr. Ann. Lyc. N. Y. **3**: 410. 1836. (As to plant described only.)" The specimen on sheet 163 of the Philadelphia copy of Part II agrees with Mackenzie's conception of *C. communis.*

CAREX OLIGOCARPA var. LATIFOLIA Gray, Gram. & Cyp. **2**: 178. 1835, nomen nudum; Torr. & Gray in Torr. Ann. Lyc. N. Y. **3**: 415. 1836. Mackenzie (N. Am. Fl. **18**: 250. 1935) cites this name in synonymy under *C. laxiculmis* as follows: "*Carex oligocarpa* var. *latifolia* A. Gray (N. Am. Gram. *no. 178* ex. syn. 1835); Torr. Ann. Lyc. N. Y. **3**: 415. 1836. (Type from Watertown, New York.)" The variety was described (Ann. Lyc. N. Y. **3**: 415. 1836) as follows: ". . . in this variety (*C. oligocarpa* var. *latifolia, Gray, Gram. & Cyp.* part 2. no. 178) the leaves are usually glaucous, the spikes more densely flowered, and the fruit usually somewhat larger." The plant on the Philadelphia sheet of no. 178 agrees with this meager description, and is certainly referable to *C. laxiculmis.*

It should also be noted that on the specimen labels, both *Festuca nutans* and *Festuca pratensis* are designated, through apparent typographical error, as number 125. However, in the index of the copy now before us, the former is listed as number 125 and the latter as number 126.

For the typification of the new species and varieties represented in this list it becomes necessary to cite a particular example of the many sets distributed, particularly since some are not homogeneous. Hitchcock and Chase (Contr. U. S. Nat. Herb. **15**: 290. 1910) designated as type of *Panicum xanthophysum* a sheet in the Gray Herbarium annotated in Gray's hand with the location "Oneida Lake, Wood Creek barrens." We should surely

regard as the type for a species one of the specimens issued in the publication in which it was described. In a later work (N. Am. Fl. **17**: 278. 1915) Hitchcock indeed names as type locality "Pine Plains, near Oneida Lake," a quotation from the printed label of No. 28 of the *Gramineae and Cyperaceae.* We here designate as types of the new species and varieties described in this work the corresponding specimens in the bound copy in the library of the New York Botanical Garden.

PAPERS IN THE ANNALS OF THE NEW YORK LYCEUM

In December 1834, Gray, who was then Torrey's assistant in the College of Physicians and Surgeons of New York, read before the Lyceum of Natural History of New York a paper entitled "A Monograph of the North American species of Rhynchospora." In this he described fifteen new species and made two new combinations; two of the novelties are based on specimens issued in the *North American Gramineae and Cyperaceae, Part I.*[9] *R. torreyana* is based on no. 96 of this work. According to personal communications of Miss Shirley Gale, the no. 96 of the bound copy in the New York Botanical Garden is actually *R. torreyana*, while the correspondingly numbered specimen at the Gray Herbarium is *R. gracilenta* (perhaps collected at a different time). *R. gracilenta* is based on no. 93 of the *Gramineae and Cyperaceae.* Our specimen again is actually *R. gracilenta*, while that at the Gray Herbarium is *R. fusca* (as labeled). This situation makes it the more imperative that specific sheets be designated as types of the species described in or on the basis of this work. Miss Gale has annotated as type of *R. torreyana* a specimen collected by Gray at Quaker Bridge, N. J., in August 1833. As type of *R. gracilenta* she has annotated a specimen, apparently collected by Gray, in the Pine Barrens of New Jersey in September 1834. Both of these specimens were formerly in the Torrey Herbarium, and are now in the herbarium of the New York Botanical Garden.

The paper on *Rhynchospora* and another read at the same time ("A notice of some new, rare, or otherwise interesting Plants, from the Northern and Western portions of the State of New York") were printed in volume 3 of the Annals of the Lyceum of Natural History of New York. In a letter to Hooker dated 4 April 1835 Gray speaks of sending the second part of the *Gramineae and Cyperaceae,* and further writes: "I inclose in the same parcel the loose sheets of an unpublished portion of the third volume of the 'Annals of the New York Lyceum of Natural History,' comprising an attempt at a monography of the genus *Rhynchospora.* A more perfect copy, with a copy of the engraving, now in the hands of the artist, will be transmitted to you by the earliest opportunity." These "loose sheets" must have

[9] Two others are published for Baldwin from his manuscript, and one for Torrey.

been printed but unbound sheets.[10] On 10 October 1836 he wrote: "All the sheets of the monograph 'Rhynchosporae' were destroyed by fire soon after being printed, and when reprinted, about a year since, I added a few observations, notes of additional localities, etc. But . . . I find there are several errors (several of which are quite material). . . . I send herewith the sheets of the paper as published here, with such typographical corrections as now occur to me." Ames (Orchidaceae **4**: 137–138. 1910) presents evidence that these pages of the Annals were finally issued in April 1836. Meanwhile, however, copies of the first printing had been distributed. Two are bound with other pamphlets ("separates") in the library of the New York Botanical Garden. One of these is of particular interest in having attached to it the original drawings from which Plate VI was made. Hooker printed the paper on *Rhynchospora* in the Companion to the Botanical Magazine (**2**: 26–38. 1836), obviously using the loose sheets of the first printing. In *North American Gramineae and Cyperaceae, Part II*, on the labels of no. 173, *Carex intumescens* var. *globularis*, and no. 185, *C. blepharophora*, the place of publication is given as "Ann. Lyc. Nat. Hist. New-York, III. p. 235." This is the pagination of the first printing, not of the second (see below). There is no doubt, therefore, that the papers were actually first issued early in 1835, and that this is the date of publication of the new names that appear in them. It is of interest to note that this original paper has been erroneously listed as a "reprint."

The pages of the 1836 version were set in a different type, which resulted in a lack of correspondence in pagination between the two editions. In the first printing the paper on *Rhynchospora* occupied pages 191–219, the second paper pages 220–236; in the second edition these pages are 191–220 and 221–238 respectively. This, with the question of the date, has led to discrepancies in the citation of certain new species and varieties. The correct references are as follows:

Rhynchospora torreyana Gray, Ann. Lyc. N. Y. **3**: 197. 1835.
Rhynchospora miliacea (Lam.) Gray, Ibid.: 198.
Rhynchospora multiflora Gray, Ibid.: 200.
Rhynchospora patula Gray, Ibid.: 201.
Rhynchospora microcarpa Baldwin ex Gray, Ibid.: 202.
Rhynchospora elliottii Gray, Ibid.: 204.
Rhynchospora corniculata (Lam.) Gray, Ibid.: 205.
Rhynchospora macrostachya Torrey ex Gray, Ibid.: 206.
Rhynchospora dodecandra Baldwin ex Gray, Ibid.: 207.
Rhynchospora megalocarpa Gray, Ibid.: 208.
Rhynchospora pycnocarpa Gray, Ibid.: 208.
Rhynchospora baldwinii Gray, Ibid.: 209.
Rhynchospora paniculata Gray, Ibid.: 211.
Rhynchospora oligantha Gray, Ibid.: 212.
Rhynchospora semiplumosa Gray, Ibid.: 212.
Rhynchospora gracilenta Gray, Ibid.: 215.
Rhynchospora cephalantha Gray, Ibid.: 218.

[10] It will be perhaps possible to verify this when the Hooker correspondence again becomes available for study. The Annals were issued in 8-page signatures, which would make it easy for unbound articles to be thus distributed.

Anemone cylindrica Gray, Ibid.: 220.
Nasturtium natans DC. var. *americanum* Gray, Ibid.: 222.
Draba incana L. var. *glabriuscula* Gray, Ibid.: 222.
Microstylis brachypoda Gray, Ibid.: 227.
Habenaria hookeri Torrey ex Gray, Ibid.: 228.
Vilfa heterolepis Gray, Ibid.: 232.
Carex intumescens Rudge var. *globularis* Gray, Ibid.: 235.
Carex blepharophora Gray, Ibid.: 235.
Aspidium acrostichoides Willd. var. *incisum* Gray, Ibid.: 236.

In the Index Kewensis, two of these species (*Rhynchospora gracilenta* and *Carex blepharophora*) are cited as of the original 1835 printing; others (*Rhynchospora baldwinii, Rhynchospora semiplumosa, Anemone cylindrica, Microstylis brachypoda, Habenaria hookeri* [as *H. hookeriana;* see Ames, l.c.], and *Vilfa heterolepis*) are listed with the pagination of the 1836 reprinting. Mackenzie (N. Am. Flora **18**: 194, 250. 1935) in citing *Carex blepharophora* and *C. intumescens* var. *globularis*, gives the pagination of the 1836 edition but the date as "1835."

From the portion of Gray's letter quoted above, it is evident that the second edition also must be mentioned in any reference to these papers. Not only were new locations added, but some descriptions were amplified or modified. Besides such important differences, many typographical errors were corrected in the second version (and some new ones introduced). It is interesting to note that Hooker's edition followed the first printing verbatim except for his own corrections of obvious errors and except for certain omissions for which he was responsible. For the convenience of those to whom both versions are not readily available, the more important discrepancies are listed below. The reader will identify some as due to typographical errors, some as resulting from Gray's annotations; about some, however, there is doubt.

1835	1836
page	*page*
196. [*Rhynchospora cymosa.*] *Corymbs* 3–4 . . the terminal one largest.	196. *Corymbs* 3–4 . . . the terminal ones largest. [So changed also by Hooker.]
	196. [dded:] Middle Florida, *Dr. Chapman.*
197. [*R. Torreyana.*] *Bristles* 6, . . . ½–3/3 the length of the nut.	197. *Bristles* 6, . . . one-half to two-thirds the length of the nut. [So corrected also by Hooker.]
197. [*R. rariflora.*] *Culms* . . . 6–12 inches high.	198. *Culms* . . . 6–12 (rarely 15) inches high.
	198. [Added:] Middle Florida, *Dr. Chapman.*
198. [*R. miliacea.*] *Lower leaves* . . . 8–16 inches long. . . . *Spikelets* . . . 1—½ an inch. . . .	198. *Lower leaves* . . . 8–10 inches long. *Spikelets* . . . ¼–½ an inch. [Hooker's correction of the latter error was: half an inch.]
	199. [Added under *R. miliacea:*] Middle Florida, *Dr. Chapman.*

page	*page*
201. [*R. multiflora.*] . . . distinguished by its much more numerous and smaller spikelets. . . .	201. . . . distinguished by its more numerous and smaller spikelets. . . .
201. I see no reason for referring this plant to the genus Scirpus.	201. [Added:] Gadsden County, Middle Florida, *Dr. Chapman.*
	201. This species produces a greater number of nuts than is usual with the genus, on which account Mr. Elliott referred it to the genus Scirpus; but *R. miliacea* and *R. caduca* often ripen nearly the same number.
	205. [Added under *R. Elliottii:*] Gadsden County, Middle Florida, *Dr. Chapman.*
206. [*R. corniculata.*] . . . one of the inner series [of bristles] about ½, the 2 others ⅓ the length of the nut.	206. . . . one of the inner series about ½, and the two others ¼ the length of the nut.
206. Ohio to Louisiana;	206. Ohio to Florida;
214. [*R. fusca.*] *Culm* . . . slender, triquetrous.	215. *Culm* . . . very slender, smooth.
217. [*R. glomerata.*] Hab. In swamps, . . .	218. Hab. In bogs and moist places;
	219. [Added under *R. cephalantha:*] Gadsden County, Middle Florida, *Dr. Chapman.*
219. [*R. cephalantha.*] . . . the bristles are hispid both upward and downward.	219. . . . some of the bristles are hispid upward and others downward.
	223. [Added under *Nasturtium natans, americanum:*] Although a rare plant, its geographical range is quite extensive, as Dr. Ingalls has recently found it at New Orleans.
223. [*Draba incana* var. *glabriuscula.*] *Flowers* in a short somewhat compact raceme, simple or rarely with one or two branches.	224. *Flowers* in a short, somewhat compact, simple (or sometimes branching) raceme.
223. The habit of our plant is precisely that of *D. arabizans Pursh, non Michx.* (Alyssum dentatum, *Nutt.* which is not an Alyssum, but a genuine Draba.)	224. The habit of our plant is precisely that of *Draba ramosissima* of Desvaux (*D. arabizans, Pursh, non Michx. D. dentata, Hook & Arn.* in Hooker's Journal of Botany. *Alyssum dentatum, Nutt.*);
224. [*Ceanothus ovalis.*] This plant is undoubtedly quite distinct from *C. intermedius, Pursh,* to which it is referred by Prof. Hooker, a species which is probably confined to the southern states.	225. This plant is undoubtedly quite distinct from *C. intermedius, Pursh,* which is merely a narrow-leaved form of *C. Americanus,* nearly confined to the Southern States.
224. [*Lathyrus palustris.*]—somewhat rare.	225. [For this is substituted:] Its leaves from lanceolate (the ordinary form) to oblong-ovate.
226. [*Dracocephalum parviflorum.*] . . . Arctic America. . . .	227. . . . British N. America. . . .
	228. [Added under *Microstylis brachypoda:*] I am not certain that these characters are constant.
227. [*Habenaria orbiculata.*] *Flowers* 15–20. . . .	229. *Flowers* 17–20. . . .
	235. [Added under *Panicum xanthophysum:*] . . . and at Burlington, Vermont, by *J. Carey, Esq.*

page	*page*
	235. [Added under *Carex chordorrhiza:*] I have recently received this plant, hitherto unknown in this country, from Seneca County, *Dr. Sartwell,* and St. Lawrence County, *Dr. Crawe.*
234. [*Carex livida.*] I have never noticed the distant or subradical peduncles. . . .	235. I have never, except in a single specimen, noticed the distant or subradical peduncles. . . .
	238. [Added under *Carex Hitchcockiana:*] Cayuga County, *J. Carey.*

Gray's next botanical work, not listed in the available bibliographies, was a further revision of the North American *Rhynchosporae* and a joint endeavor with Torrey on the *Carices* of North America. These were printed as a part of Torrey's *Monograph of North American Cyperaceae* (Ann. Lyc. Nat. Hist. N. Y. **3**: 239–443. 1836.). In his introductory remarks, Torrey wrote: "The revision of the Rhynchosporae is entirely his [Gray's] own; and the Synopsis of North American Carices, I wish to have considered as our joint performance." The correct citations for species or combinations published in these portions of the "Monograph" have already been discussed by one of us.[11]

THE NEW YORK BOTANICAL GARDEN
NEW YORK, N. Y.

[11] C. Gilly, A note on the authorship of certain species of cyperaceae. Rhodora **43**: 333–335. 1941.

INDEX TO AMERICAN BOTANICAL LITERATURE

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Papers that relate exclusively to bacteriology, forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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(See also under Plant Physiology: **Alexopoulos & Herrick; Robbins & Kavanagh;** under Morphology: **Fulling**)

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HERBERT MCKENZIE DENSLOW

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Herbert McKenzie Denslow was born at Lynn, Massachusetts, August 20, 1852. As a boy he became interested in botany through association with his uncle, William Wallace Denslow (1826–1868). Together they collected plants, often in the wilds of upper Manhattan; both were among the original members of the Torrey Botanical Club, in 1867. The uncle died shortly after this time, but the nephew has maintained his botanical interests throughout the 75 years of the Club's existence.

Dr. Denslow graduated from Yale in 1873. He was given the degree of A.M. by Kenyon College in 1896, and that of D.D. by General Theological Seminary in 1907. In 1878 he became a deacon, in 1879 a priest in the Protestant Episcopal Church. From 1878 to 1902 he discharged the duties of a clergyman at various places in Connecticut, Vermont, western New York, Ohio, and Indiana. During these 24 years he was unable to take active part in the meetings of the Torrey Club; but he maintained the connection as corresponding member. In 1902 he returned to New York as Professor of Pastoral Theology in General Theological Seminary, in which capacity he served until his retirement as Professor Emeritus in 1923. For a part of this period he was sub-dean, and at times acting dean.

Dr. Denslow's botanical work has been particularly concerned with our native orchids. A considerable list of publications bears witness to his attainments in this field, and in his search for these plants he has visited nearly every town in the state of Connecticut. His studies have been intensified during his late years, since retirement from professional duties.

To all who enjoy the privilege of his acquaintance, he is outstanding as a profound yet tolerant scholar, and as a kindly and cordial gentleman. The Club honors itself in congratulating its oldest living member on his ninety years.

J.H.B.

The portrait of Dr. Denslow is published with the assistance of the Lucien M. Underwood Memorial Fund.

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NUCLEAR BEHAVIOR IN THE MUCORALES—I. THE MUCOR PATTERN

VICTOR M. CUTTER, JR.

For the past 60 years the question of nuclear behavior in the Mucorales has been a source of controversy. The exact stages in the life history at which nuclear fusion and the subsequent nuclear reduction take place have never been satisfactorily demonstrated. It is obvious that before further studies in the sexuality and genetics of this group can be carried out, these critical stages must be studied from a comparative basis and their position definitely established. A survey of the 50-odd publications dealing with this question indicates that the present confusion arises from four principal sources. The extremely minute nature of the mucoraceous nucleus, the use of unsatisfactory cytological techniques, the great natural variation in the material used in these investigations, and the failure of the majority of workers to follow the nuclear behavior through all the stages in the life history have all contributed to this controversy. This and a subsequent paper present the results of a correlated study of the nuclear condition in 15 species of this group at all stages in the life cycle.

MATERIALS AND METHODS

The species used in this study are listed below. An attempt has been made so far as possible to select species which have been studied previously in order that the results of the present investigation might be reasonably compared with those of earlier investigations, and also in the hope of correlating some of the divergent opinions on controversial points. The nomenclatural system followed is that of Naumov (21). Herbarium material of all the species studied has been deposited in the Herbarium of Plant Pathology at Cornell University and in the Farlow Herbarium of Cryptogamic Botany at Harvard University under the numbers indicated in the accompanying table.

Species investigated

No.	Species	No.	Species
25	*Mucor genevensis* Lendner	87	*Zygorhynchus vuillemenii* Namysl.
82	*Mucor hiemalis* Wehmer	90	*Zygorhynchus vuillemenii* var. *agamus* Namysl.
13	*Parasitella simplex* Bainier	7	*Absidia spinosa* Lendner
63	*Zygorhynchus dangeardi* Moreau	81	*Blakeslea trispora* Thaxter
68	*Zygorhynchus moelleri* Vuill.		

In order to secure as much uniformity as possible all material was cultured upon a single substrate, carrot decoction agar. Mature zygospores were

germinated by sterilizing them in 12 per cent calcium hypochlorite solution and isolating individual spores on 2 per cent non-nutrient agar, or moist filter paper in petri dishes after they had reached the proper age. A future publication concerning the details of this process and the results of a number of experiments upon the factors influencing germination of the zygospores of the Mucorales is anticipated.

Young mycelia, sporangia, and the early stages in zygospore formation were prepared for cytological examination by the method recommended by Schwarze (26) in his study of the water molds. The same technique was used in preparing the germ tubes of zygospores for sectioning. Older zygospores, chlamydospores, and hyphal bodies were lifted from the substrate attached to portions of the mycelium and immediately immersed in fixative. In such cases an aspirator was used to remove all air from the material, and to insure thorough penetration of the fixative. In this connection a detergent "Tergitol" Penetrant 7, supplied by the Carbide and Carbon Chemicals Corporation of New York was used in conjunction with all fixatives. The addition of three drops of this compound to every 50 cc. of the fixative was found to lower the surface tension of the solution to the point where almost instantaneous wetting of the material occurred. In addition, the penetration of the fixative into larger thick-walled structures was greatly enhanced.

As might be expected the most nearly natural and lifelike fixation images were produced by the various chromic-osmic-acetic mixtures, of which Flemming's weak solution and Chamberlain's solution as cited by Johansen (13) proved the most useful. Randolph's modification of Navashin's fluid cited under the abbreviation Craf (13) has proved excellent in studying the older zygote stages where it is difficult, because of excess reserve substances, to bleach out the osmic acid after fixation. Formalin-acetic-alcohol is very useful for the study of nuclear structure in young hyphae and zygospore stages. Gilson's fluid (13) and saturated mercuric chloride solution have been extensively used in conjunction with the Feulgen reaction. With this procedure they prove quite satisfactory but are practically worthless when followed by other stains.

For a study of the chondriome and of the cytoplasmic inclusions two solutions developed by Zirkle (31) have given the best results. Zirkle-Erliki fluid removes all chromatin and preserves mitochondria well, while Zirkle's reduced chromic fluid preserves vacuoles and mitochondria well and at the same time does not completely obliterate the chromatin present, so that a combined picture of the acid and basic images may be secured. Regaud's fluid as cited by Lee (16) occasionally gave satisfactory results but it was troublesome to use with the older stages and was not so generally employed as the two preceding formulae.

Dehydration of all materials, before they were embedded in paraffin, was carried out in the n-butyl alcohol series given by Zirkle (31). This method

proved much more satisfactory than did any of the xylene or chloroform series and tended to reduce shrinkage to a minimum. With the older zygospore stages it was necessary, because of the brittle nature of the exospore, to subject the material to a softening process before sectioning. The best results were obtained with material soaked in 3 per cent sodium hydroxide immediately after fixation. All materials were embedded in 56° paraffin, and sections cut from 3 to 15 microns thick.

It became obvious early in the course of this study that little would be gained by repeating the staining methods usual to this type of investigation, since the unsatisfactory results obtained by many workers indicated that Flemming's triple stain and Heidenhain's haematoxylin were not sufficiently precise to reveal nuclear detail during the stages when various structures were in the dormant condition. Hence a definite attempt was made to adapt more modern and precise staining techniques to these fungi. Several staining methods not previously employed in the study of the Mucorales have been used. The first of these and perhaps the most important was the Feulgen reaction. Great care must be used in the interpretation of structures showing a reaction to this test, for, as Knaysi (15) points out, the significance of this reaction is as yet not fully understood. The staining solution was prepared in accordance with Coleman's (7) modification of de Tomasi's method. The time of hydrolysis varies with the fixative employed, and in this connection Hillary's (12) schedule has been followed with very satisfactory results.

The second staining method used was based upon a technique described by Naylor (22) but different dyes and a different buffer series was used. The dyes selected were 0.5 per cent aqueous erythrosin and saturated aqueous methylene blue as suggested by Savile (25). His staining schedule was followed but the buffer selected was the acid potassium-phthalate series of Clark and Lub (6). This buffer was employed in place of the disodium-phosphate citric acid series recommended by Savile, because of the difficulties encountered with fungi growing in the latter solutions.

Hruby's modification of the Cahal-Brozek basic fuchsin-indigocarmin method (13) has proved to be a very rapid and practical stain for mycelium and young zygote stages in conjunction with any fixative. Samples of all the material stained by the three preceding methods were also stained in Heidenhain's iron alum–haematoxylin and in Flemming's triple stain for purposes of comparison. In all cases the results obtained by the newer techniques were equally satisfactory, and with respect to the stages in the life cycle where abundant reserve substances were present in the fungus structures these methods gave more precise results. For a study of the elements of the chondriome, staining with Heidenhain's iron alum–haematoxylin, and the mitochondrial method of Bensley-Cowdry cited by Lee (16), have proved generally satisfactory.

The illustrations in this paper have been made diagrammatic to the extent that no detail has been rendered in the cell walls, since this has no bearing on the present problem. However, important dimensions such as the relative thickness of the several zygospores wall layers have been portrayed as accurately as possible. The exospore has been shown in black, and the endospore has been left blank to secure a contrast between these two walls. Amorphous structures, such as oil vacuoles, have been left blank to distinguish them from the reticulately stippled cytoplasm. All drawings were made with a projection mirror or camera lucida adjusted to table top level and with Leitz apochromatic objectives and compensating eyepieces. Inasmuch as other workers on the same problem have figured many of the stages encountered in this study, no attempt has been made to reillustrate those stages which have previously been adequately portrayed.

Abbreviations. The following abbreviations of stains and fixatives are used in the legends accompanying the figures, to indicate the type of preparation from which the drawings and photographs were made.

Fixatives

Flemming's weak solution	Flemming's
Chamberlain's solution	Chamberlain's
Gilson's fluid	Gilson's
Saturated mercuric chloride	S.M.C.
Formalin-acetic-alcohol	F.A.A.
Randolph's modification of Navashin's fluid	Craf
Zirkle-Erliki fluid	Zirkle's
Zirkle's reduced chronic fluid	Z.R.C.

Stains

Feulgen reaction for nucleoprotein	Feulgen
Methylene blue and Erythrosin used with buffer solutions	Buffer
Cahal-Brozek basic fuchsin indigocarmin	Cahal-Brozek
Bensly-Cowdry acid fuchsin light green for mitochondria	Bensly-Cowdry
Flemming's triple stain	Triple
Heidenhain's iron-alum haematoxylin	Haematoxylin

Certain matters pertaining to the terminology of the various nuclear and cytoplasmic structures require clarification before proceeding to a detailed discussion of the species investigated. Baird (2) has presented a very complete review of the investigations on nuclear structure and consequently such a summary will be omitted here. As interpreted in the following study, a typical vegetative nucleus in the Mucorales consists of one or more deeply staining central bodies surrounded by a clear region which may or may not be bounded by an obvious membrane. Around the periphery of this clear region and within the membrane, if one is present, a limited number of chromatic threads are arranged indiscriminately. These threads rarely form a definite reticulum, and are only visible after very precise fixation. At the

present time it is impossible to state whether they represent the complete nuclear reticulum or are merely the basichromatic portions of a more extensive reticulum which has, as yet, not been demonstrated. These threads are Feulgen positive and have been found in all the metabolic nuclei examined. A nuclear membrane is apparently associated with all metabolic nuclei, although the small size of the nuclei and the fact that the membrane frequently collapses under the action of fixatives frequently make it difficult to detect.

The central body is the most prominent constituent of the nuclear unit. It has commonly been referred to as a nucleole or nucleolus, but by some authors (2) it has been interpreted as the entire nucleus. Whether this body can be considered homologous with the nucleolus of higher organisms is open to question. It is highly refractive and stains brilliant red with Flemming's triple stain in contrast to the bluish or purple reticulum; on this basis it has been considered a typical nucleolus. On the other hand in material subjected to the Feulgen reaction the central body is Feulgen positive, as is also the nuclear reticulum. During division it separates into two approximately equal portions and passes to the poles of the spindle in company with the chromatic elements of the reticulum. These facts would mitigate the nucleolus theory and perhaps suggest that the mucoraceous nucleus shows affinities with the karyosome nuclei of certain lower organisms (27). It is conceivable, however, that the reaction to the Feulgen test results from the collopse of a Feulgen positive portion of the reticulum around the central body; thus masking its true reaction. Until further light is thrown upon this matter it seems advisable to refrain from designating this structure the nucleolus, since this would imply that it is homologous with the nucleolus of higher organisms.

In the species considered below two types of nuclear division occur. In the rapidly growing portions of the thallus mitotic divisions take place. During division a very faint spindle is developed with the highly chromatic mass of the central body and presumably also of the nuclear reticulum lying in the equatorial region. The nuclear membrane becomes very faint at this stage but does not completely disappear: hence the achromatic figure may be considered intranuclear. The chromatic mass then separates into two approximately equal portions which move towards the poles of the spindle. As these masses reach the poles, a clear region forms around each of them, the spindle disappears and shortly thereafter a nuclear membrane is again apparent around each daughter nucleus. At no stage in this process has it been possible to distinguish definite chromosomes, although the chromatic masses during their migration to the poles are not entirely homogeneous. Occasionally during the early division stages refractive granules can be seen at or near the poles and outside the nuclear membrane. These structures probably represent centrioles, but their behavior before the onset of nuclear division and

after the reorganization of the daughter nuclei could not be determined. They are by no means universally present in these fungi as Moreau's account (18) might lead one to believe. The periodicity of mitotic division was very marked in all the forms examined and frequently it was possible to investigate large amounts of material without encountering any division stages.

In the older vegetative mycelium and the maturing and dormant zygospores a type of nuclear division which is perhaps best interpreted as amitosis occurs. Here the central body of the nucleus merely elongates, the nuclear membrane disappears, and the chromatic masses are divided by constriction into two or more portions which move apart. In a few cases the divided chromatic masses became associated with new nuclear membranes, but it is not clear whether this is always the case. Figures of this type are common in the portions of the thallus where active cytoplasmic movement has ceased. Inasmuch as there are frequently large amounts of reserve substances present at these stages, the question arises whether these presumably amitotic figures do not, in reality, represent typical mitotic figures in which the finer details of the achromatic figure are obscured by ergastic materials. On the other hand this type of division may represent the first stages in the degeneration of nuclei which have become superfluous.

The results of this study indicate that nuclear degeneration occurs in some cases, but no evidence has been found that it always occurs at the same stage. The phenomenon may also be exhibited after improper fixation, which may lead to erroneous conclusions regarding its frequency. Nothing was seen to indicate that the failure of nuclei to fuse in the zygospore necessarily leads to ultimate degeneration of the unfused nuclei. In certain species unfused nuclei appear to persist throughout the life cycle.

Both vegetative and sexual fusions of nuclei have been frequently reported in this group. The significance attached to this phenomenon seems largely a matter of interpretation. The obvious difficulties in distinguishing between late stages in nuclear fusion and early stages in nuclear division where an achromatic figure cannot be demonstrated are manifest. For this reason only those cases in which nuclei with membranes intact have been observed in the process of fusion have been regarded as having any biologic significance. The fusion process usually results in a marked increase in size of the fusion nucleus. It has never been possible to demonstrate any fundamental difference in the fusing nuclei, or to trace their possible parentage. From analogy with other forms, however, it seems reasonable that only nuclei of different genetic composition undergo karyogamy. Fusion takes place at different stages in the zygospore of the several species studied, and no evidence has been found of the reported vegetative karyogamies in the mycelial stages.

The results of many workers indicate that the nuclei of the vegetative

mycelium and the zygospores are not always of the same size or appearance at different stages. In regions where active cytoplasmic movements are going on the nuclei are usually larger with a well defined membrane and nuclear reticulum, whereas in regions where the growth rate is retarded or has ceased the nuclei tend to be contracted and the nuclear membrane difficult to demonstrate. This size difference which is correlated with cytoplasmic activity can be noted in both fused and unfused nuclei. Since these two phases are so characteristic it has seemed well to apply the terms "expanded nuclei" and "unexpanded nuclei" respectively to describe the two conditions. These noncomittal terms have been purposely selected since they imply no fundamental difference in the nuclear structure. The two phases grade into one another in transitional regions. The actual size of the nucleus varies greatly in different species. This change in nuclear size which is neither the result of karyogamy nor of nuclear reduction has not been clearly recognized in earlier investigations, and it is of considerable importance in the proper interpretation of nuclear behavior in the zygotes.

During meiosis the fusion nuclei are larger than the unfused nuclei and are much more prominent in the cell. In the meiotic divisions chromosomes are clearly delimited and are of considerable size. The chromosome number in those species in which it has been determined is 12 in the haploid condition. Clear meiotic figures have not been seen in all the species studied; but it has been possible, owing to the considerable size difference between fused and unfused nuclei, and by analogy from those species in which reduction division has been demonstrated, to locate the approximate point in the life cycle where this process takes place in all the species included here. The expanded and unexpanded nuclear phases referred to above must be taken into consideration when the criterion of nuclear volume is used to distinguish haploid from diploid nuclei, since both fused and unfused nuclei enter these characteristic phases.

An investigation of the cytoplasmic constituents of the thallus of these forms by the use of basic fixatives and cytoplasmic stains has revealed, in addition to the various reserve substances usually present, two types of structure which seem to fulfill the definition of mitochondria given by Newcomer (23). These bodies are preserved by bichromates between a pH range of 4.6 and 5.0. In rapidly growing hyphal tips and in the young progametangia they appear in the form of long, very thin threads. In the zygospores and chlamydospores, just prior to the formation of the prominent reserve substances, there are present very minute granules which exhibit the same staining reaction as these threads. In all probability these structures are homologous as indicated by their response to different reagents and by the intermediate forms sometimes encountered.

Numerous workers have reported the presence of mucorine crystals in the

older mycelium and zygospores of these forms. A survey of the literature on the question conveys the impression that these structures are always present at some stage or other in the life cycle. The present results indicate that under many conditions these crystals are not developed at all. Ling Young (17) has presented an extensive account of the formation and composition of these crystals and nothing can be added to his observations. The presence of amorphous bodies apparently concerned with the secretion of oil has been frequently pointed out. Keene (14) and Moreau (19) have discussed these bodies and speculated as to their origin. In general the observations of these two investigators on this category of ergastic substances is confirmed, although additional evidence indicating that the mitochondria may function in their formation is presented below. Keene's usage of the term "oil plastids" for these structures will be followed.

OBSERVATIONS

MUCOR GENEVENSIS Lendner. This homothallic species falls in the section *Hagemia* of the genus *Mucor* in the classification of Naumov (21). A number of species in this section have been investigated by various authors most of whom are in agreement concerning the nuclear behavior in the younger zygote stages. Dangeard (8) examined *Mucor fragilis* Tode, a species excluded by Naumov, and probably synonymous with *M. hiemalis* Wehmer, and observed nuclear fusions in the developing zygospore. The nuclei which failed to conjugate apparently disintegrated. In the azygospores he did not encounter any evidence of nuclear fusion or degeneration; the nuclei remaining unchanged throughout the development of the azygospores. Moreau (19) reports nuclear fusions, which he interprets as karyogamies, during the period when the exospore of the zygote is being deposited, in *M. sylvaticus* Hagem, *M. hiemalis* Wehmer and *M. genevensis* Lendner. Degeneration of unfused nuclei then occurs. Ling Young (17) working with *M. hiemalis* and *M. genevensis* corroborates Moreau's observations but does not extend them. None of these workers followed the nuclear condition through the dormant period of the zygospore, nor did they observe germination of the zygospore.

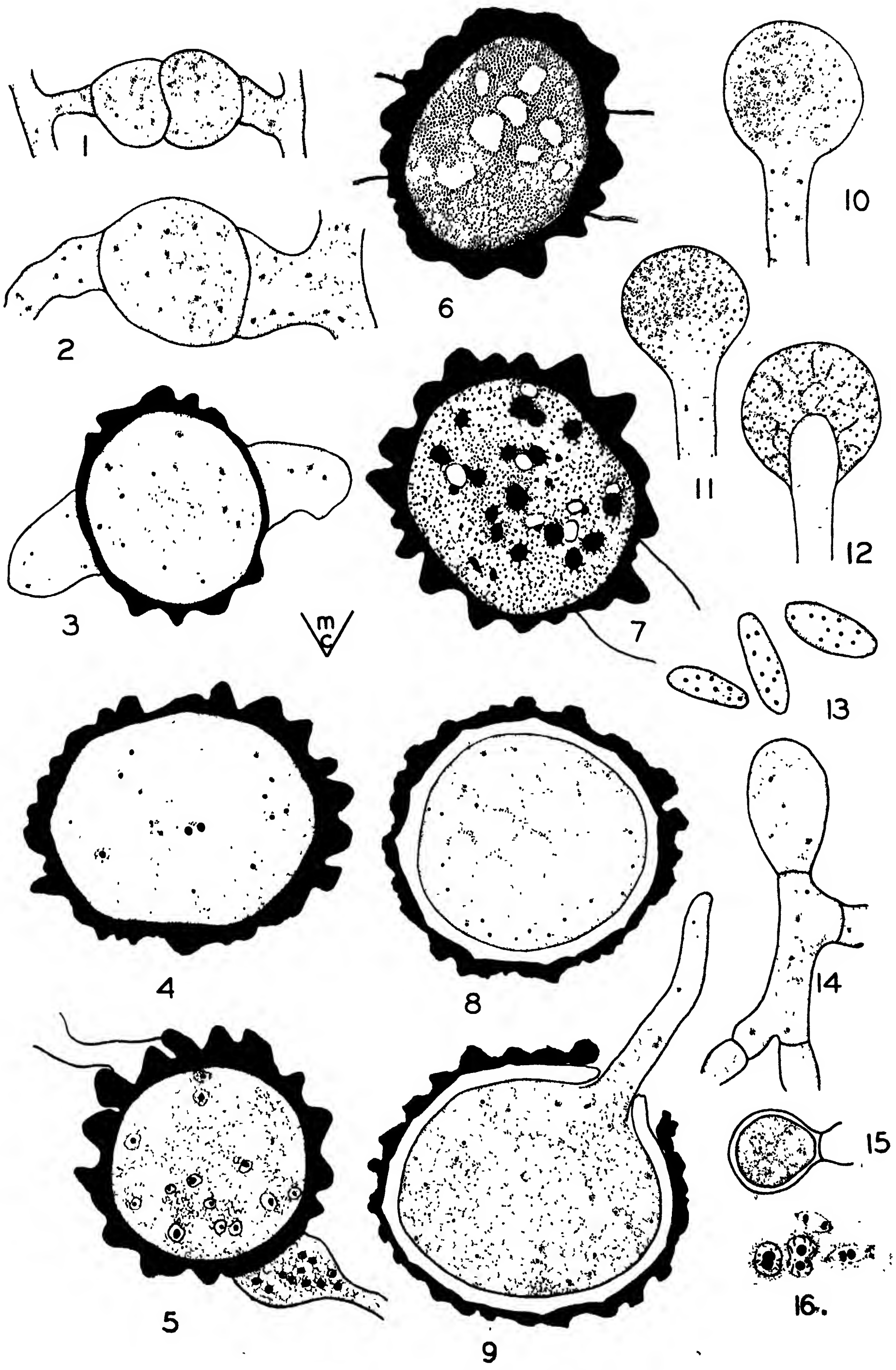
The present study confirms, in general, the observations of these workers on the early stages of zygospore development. The progametangial initials are multinucleate and mitotic divisions are in evidence as they increase in size (fig. 1). The number of nuclei in the young gametangia is not large. Nuclear division ceases as the coenozygote is formed, and perhaps fifty nuclei are present in the average coenozygote (fig. 2). These nuclei are arranged indiscriminately in the cell, and as the exospore matures they show a paired arrangement and a marked increase in size (fig. 4). As the exospore assumes its mature form, very distinct nuclear fusions are in progress (fig. 61). In all the material examined the great majority of nuclei in the cell

fused in pairs, and only an occasional unfused nucleus could be discerned in zygospores 4 days old (figs. 5, 62). These fusion nuclei persist in the expanded state for several days during which time oil plastids become very numerous in the zygospore, and the cytoplasm becomes quite dense. On about the sixth day of development the endospore is differentiated and almost simultaneously with the appearance of this structure a distinct change in the size and number of nuclei in the zygospore takes place (fig. 8). In place of the few large expanded fusion nuclei previously in evidence, there are now apparent about twice as many smaller nuclei, which remain in the expanded state for only a short time and then rapidly sink into the unexpanded phase with the onset of dormancy in the zygote. From analogy with the situation described below in *Absidia spinosa,* where the nuclear behavior is similar to that of *M. genevensis* and where, apparently, the meiotic divisions occur at this stage, it appears reasonable that this change in nuclear size and appearance is the result of a reduction division of the fusion nuclei. By the end of the eighth day after the formation of the coenozygote, most of the zygospores have entered the dormant condition. The resting period persists for about 4 months and no further change in the nuclear condition can be seen. The cytoplasm gradually contracts into the peripheral regions of the spore and the oil plastids are very prominent, tending to obscure all the other cell constituents.

Zygospores of this species are capable of germination approximately four months after their formation and remain viable until the end of the seventh month. At the onset of germination the oil reserve disappears, the cytoplasm becomes vacuolate and rapid mitotic divisions take place which differ in no way from those seen in the mycelium and progametangia. The zygospore walls are soon ruptured and a thick germ tube is pushed out (fig. 9). At this stage the exospore wall becomes quite translucent and germinating zygospores can be easily distinguished from those still in the dormant con-

Explanation of figures 1–16, Mucor genevensis

FIG. 1. Progametangia in contact (Flemming's; Haematoxylin). ×660. FIG. 2. Coenozygote 7 hours old (Flemming's; Haematoxylin). ×660. FIG. 3. Zygospore 15 hours old. Dark region in center may represent coenocentrum (Flemming's; Cahal-Brozek). ×660. FIG. 4. Zygospore 2 days old; fused and unfused nuclei present; central nuclei undergoing syngamy (Chamberlain's; Haematoxylin). ×660. FIG. 5 Zygospore 5 days old with fusion nuclei in expanded phase (Gilson's; Feulgen). ×660. FIG. 6. Zygospore 4 days old showing mitochondria and formation of oil plastids (Zirkle's; Haematoxylin). ×660. FIG. 7. Zygospore 5 days old, showing chondriome and oil plastids; the clear areas probably represent nuclear vacuoles from which the chromatin has been dissolved (Z.R.C.; Bensly-Cowdry). ×660. FIG. 8. Dormant zygospore; nuclei in unexpanded phase (S.M.C.; Feulgen). ×660. FIG. 9. Germinating zygospore 4 months old (Flemming's; Triple). ×660. FIGS. 10–12. Sporangium development (Flemming's; Haematoxylin). ×660. FIG. 13. Mature sporangiospores; nuclei unexpanded (F.A.A.; Buffer). ×1000. FIG. 14. Hyphal bodies and young terminal chlamydospore (Flemming's; Cahal-Brozek). ×660. FIG. 15. Terminal chlamydospore 6 days old (F.A.A.; Buffer). ×660. FIG. 16. Stages in nuclear fusion and mitotic division in zygospore 3 days old (Flemming's; Haematoxylin). ×1350.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dition. The germ tube develops into a single unbranched sporangiophore terminated by a germ sporangium. The sporangiospores of this germ sporangium all give rise to homothallic mycelia.

Particular attention has been given to the nature of the chondriome in this species. In the hyphal tips of the mycelium and in developing sporangia long, extremely delicate, thread-like mitochondria are arranged in a loose weft in the regions of active cytoplasmic movement. As the cytoplasmic activity ceases in the older portions of the mycelium, these structures are no longer discernible, but minute granules can be demonstrated by the same fixing and staining methods. These mitochondria become particularly abundant in the regions where accumulation of reserve products takes place. They are very prominent in developing chlamydospores and in the younger stages of the coenozygotes, where they are arranged in a regular reticulum. They persist in this condition until the appearance of the oil plastids which seem to arise by a coalescence of these granules (figs. 6, 7, 65). The oil plastids exhibit the same staining reactions as the mitochondria, but this might be attributed to the masking effect of many mitochondria aggregated over the surface of the plastids. In the stages where the oil plastids are fully developed, mitochondria can no longer be demonstrated as distinct units. They have either disappeared or have been incorporated into the oil plastids. In the germinating zygospores, where the oil plastids and most of the reserve substances have disappeared the granular mitochondria have not been observed, but the thread-like forms are again apparent in the active cytoplasm at the tip of the germ tube.

The formation of the sporangiophores and sporangia of this species follows in the early stages the same course which Moreau (19) describes for *Mucor spinescens*. The sporangiophores are usually unbranched, and at their tips terminal swellings develop, into which a considerable amount of cytoplasm is carried. Mitotic nuclear divisions take place with great rapidity in the sporangial fundament (fig. 10). As the fundament reaches its maximum size the cytoplasm becomes differentiated into a peripheral sporogenous layer which contains most of the nuclei and an internal more or less sterile region which ultimately becomes the site of the columella. This stage is of short duration and then numerous flattened vacuoles appear in the fertile zone and divide the cytoplasm into many multinucleate blocks (fig. 11). These blocks may contain as many as 15–20 nuclei. At the same time a broad vacuole is delimited on the site which the columella membrane is to occupy. These vacuoles are filled with a refractive hyaline intersporal substance, which appears to function in the deposition of the sporangiospore walls and the columella membrane. After the appearance of the intersporal substance the multinucleate cytoplasmic segments contract somewhat and without further subdivision assume the characteristic elongated shape of the spo-

rangiospores. They then acquire a spore wall, and at the same time, a heavy wall which becomes the columella is deposited around the lower edge of the broad vacuole which separates the fertile from the sterile region (fig. 12). It must be emphatically stated that the columella does not arise by the upward burgeoning of the septum which is usually formed below the sporangial fundament. The few nuclei present in the columella soon revert from the expanded to the unexpanded phases and amitotic divisions may occur. The cytoplasm becomes very vacuolate and soon contracts against the walls of the sporangiophore, or becomes incorporated into chlamydospores which in some strains of this fungus develop luxuriantly in the sporangiophores. It will be noted that the process of sporangiospore formation in this species differs from that described by Moreau in *M. spinescens* in that the subdivision of the sporogenous cytoplasm ceases at an earlier stage, and this cytoplasm does not become arranged into cords before the spore initials are delimited. This is presumably a more primitive condition than that in *M. spinescens* since reduction in spore size and nuclear number is not carried so far (fig. 13).

Chlamydospores are very abundant in this species and in some strains the submerged mycelium may be almost completely transformed into these bodies. In newly formed chlamydospores (fig. 14) the nuclei are generally in the expanded condition and very occasionally mitotic divisions can be seen, but the nuclei enter the unexpanded phase as soon as the reserve substances begin to accumulate. Oil plastids of the same type as those seen in the zygospores are found in the chlamydospores and the same relationship between oil plastids and mitochondria has been observed.

MUCOR HIEMALIS Wehmer. This is a heterothallic species very closely related to *Mucor genevensis,* previously investigated cytologically by Moreau (19) and Ling Young (17). Both these authors find that in all stages investigated by them the nuclear behavior is completely similar in both species. In the strain of *M. hiemalis* used here all the sexual and asexual stages investigated were entirely similar to the corresponding stages in *M. genevensis* as far as nuclear behavior was concerned, and the species could not be distinguished cytologically.

The problem of sex segregation in this form presents certain peculiar aspects. The sporangiospores of any given germ sporangium produce, upon germination, mycelium which is entirely of one sex or the other. To date no sporangia have been found which contain spores of both sexes. In other words, segregation of sex is complete at the time of reduction division. This is unusual in that no plausible explanation has presented itself why only spores of one sex should persist at the expense of spores of the other sex in any given germ sporangium. The conditions affecting this phenomenon are as yet problematical and further investigation is necessary.

PARASITELLA SIMPLEX Bainier. This species, encountered as a parasite upon various genera of the Mucorales, resembles very closely some of the species in the *Hagemia* section of *Mucor*. Taxonomically it is particularly close to *M. hiemalis* Wehmer. From these it can be distinguished by its habit of forming curious galls or "sikyospores" upon the hyphae of species of the Mucorales with which it comes in contact. Burgeff (5) and Satina and Blakeslee (24) have investigated this phenomenon in detail. Burgeff has also carried out a cytological examination of the nuclear behavior in these galls. As far as can be determined, the nuclear condition has not been followed through the development of the zygospore of *Parasitella,* although Burgeff has discussed the development of the zygospore from a morphological point of view.

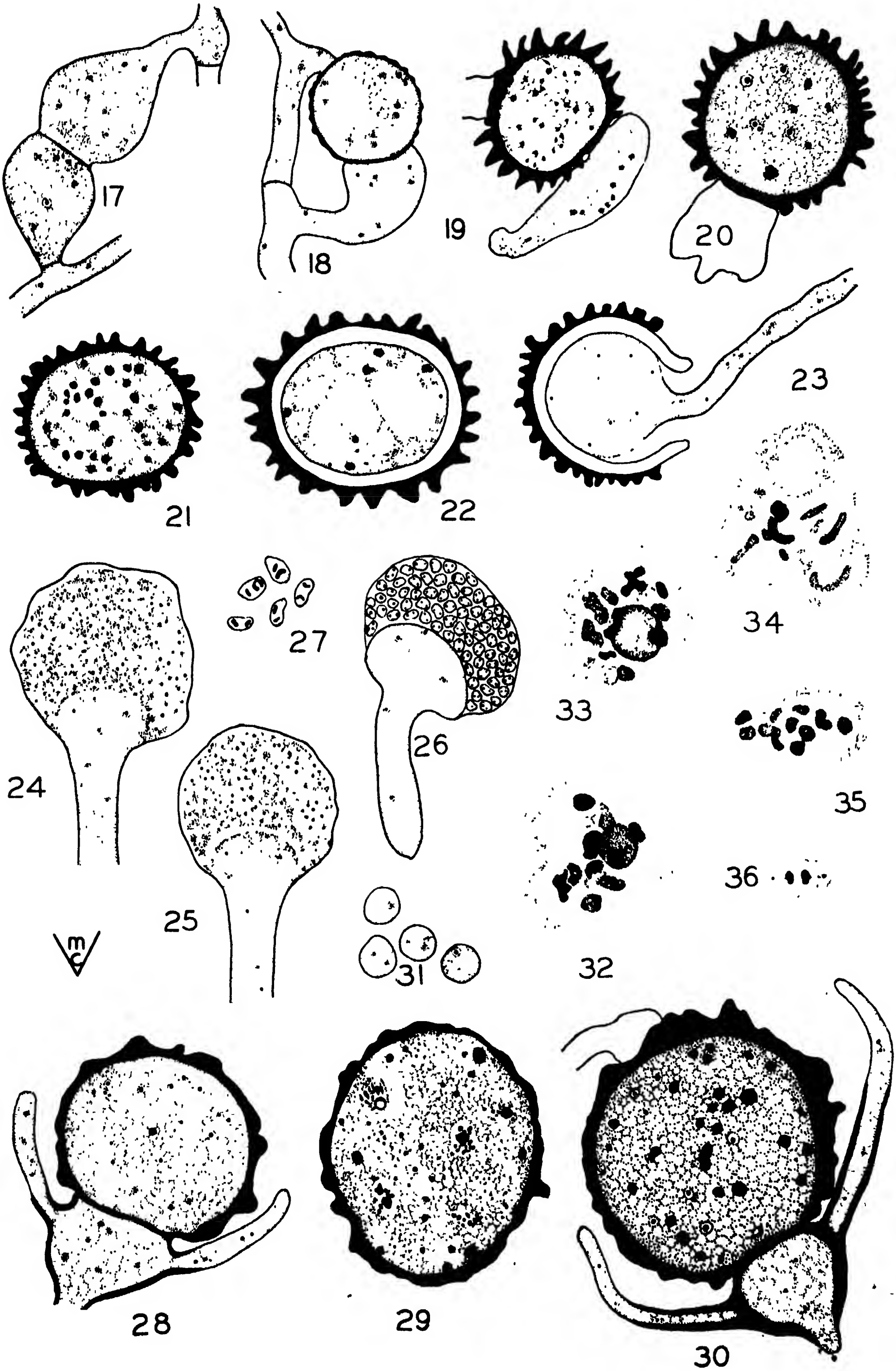
In our material of *Parasitella* zygospores were formed in small numbers, and were in all respects identical to those described by Burgeff, with the exception that in our cultures no spores were found bearing the *Absidia*-like outgrowths from their suspensors which Burgeff has illustrated for some of his material.

The nuclear condition in this strain of *Parasitella* differs in no way from that previously described in *M. genevensis* and *M. hiemalis.* Germination of the zygospores proceeds along the same pattern and occurs within the same time range. *Parasitella simplex* differs in only a few minor morphological details from *M. hiemalis* and cannot be differentiated, in our strains at least, on a cytological basis.

ZYGORHYNCHUS DANGEARDI Moreau. A detailed review of all the literature pertaining to *Zygorhynchus* will not be attempted, since Green (9) has presented a comprehensive account of the progress of this work up to 1927. The

Explanation of figures 17–27, Zygorhynchus dangeardi; and of figures 28–36, Absidia spinosa

FIG. 17. Progametangia in contact (Chamberlain's; Cahal-Brozek). × 660. FIG. 18. Zygospore 8 hours old (Flemming's; Haematoxylin). × 660. FIG. 19. Zygospore 15 hours old, some nuclei degenerating (Flemming's; Triple). × 660. FIG. 20. Zygospore 3 days old; fusion nuclei in expanded phase; oil plastids developing (Flemming's; Haematoxylin). × 660. FIG. 21. Zygospore 6 days old; cytoplasm becoming vacuolate and oil plastids numerous (Craf; Haematoxylin). × 660. FIG. 22. Dormant zygospore; nuclei unexpanded (F.A.A.; Buffer). × 660. FIG. 23. Germinating zygospore 78 days old; mitotic divisions in germ tube (Flemming's; Triple). × 660. FIGS. 24–26. Sporangium development (Flemming's; Triple). × 660. FIG. 27. Sporangiospores 2 weeks old; nuclei unexpanded (F.A.A.; Buffer). × 1000. FIG. 28. Zygospore 16 hours old, unfused nuclei in expanded phase (Flemming's; Feulgen). × 660. FIG. 29. Zygospore 7 days old; 5 fusion nuclei in prophase of first meiotic division; oil plastids developing (Craf; Haematoxylin). × 660. FIG. 30. Zygospore 3 days old; fusion nuclei in expanded phase; oil plastids numerous (F.A.A.; Cahal-Brozek). × 660. FIG. 31. Sporangiospores (F.A.A.; Buffer). × 1000. FIGS. 32–34. Prophase stages of first meiotic division in zygospores 7 days old; the large nucleolus-like bodies probably represent developing oil plastids (Craf; Haematoxylin). × 3100. FIG. 35. Metaphase of first meiotic division (Craf; Haematoxylin). × 3100. FIG. 36. Anaphase of mitotic division in vegetative mycelium (Craf; Haematoxylin). × 3100.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only important contribution which has appeared since that time is Ling Young's (17) account of the nuclear behavior in *Z. moelleri, Z. macrocarpus,* and *Z. dangeardi* in 1930. No attention will be given to the morphological development of the fungus, this having been adequately described by Atkinson (1), Blakeslee (3), Green (9), and other workers.

The nuclear behavior in *Z. dangeardi* has been discussed by Moreau (19). He points out that it differs from its close relative, *Z. moelleri* Vuill., in that the number of fusion nuclei in the zygospore is greatly reduced. In some cases there are only four fusion nuclei present in the zygospore prior to the onset of the dormant condition. Nuclear fusion also takes place at a later stage in zygospore development than in *Z. moelleri* Vuill. In *Z. macrocarpus* Ling Young, which according to its author (17) resembles *Z. dangeardi* very closely in its morphological characters, the nuclear behavior follows the same pattern as that in *Z. moelleri;* that is to say the number of fusion nuclei are not particularly reduced as in *Z. dangeardi.*

In the zygophores and young progametangia of the present strain of *Z. dangeardi,* expanded, unfused nuclei are present in fair numbers. It should be emphasized, however, that the cytoplasm of all the species of *Zygorhynchus* does not possess the extremely large number of very minute nuclei which are characteristic of such genera as *Rhizopus, Phycomyces,* and *Sporodinia.* The nuclei are also somewhat larger in proportion to the thallus size than in the aforementioned genera. As the progametangia develop, and the suspensors are delimited by the typical cleavage furrow which develops in the form of a closing diaphragm, sporadic mitotic divisions are apparent. The nuclei in these young sex organs do not, however, become proportionately more numerous than in the zygophores (fig. 17). In the young coenozygote the number of nuclei is not large, and there is no evidence that any nuclear degeneration has yet occurred. As the exospore matures, nuclei may be observed which are fused, associated in pairs, or unfused. All nuclei are still in the expanded condition. Nuclear fusions occur until about the sixth day after the appearance of the exospore when apparently all the nuclei which entered the young coenozygote have fused in pairs (fig. 20). No indication of the degeneration of any nuclei has been observed in this species. The number of fusion nuclei present varies with the size of the spore, but is never large. On the average 10–12 fusion nuclei are present in most zygospores at this time.

Oil plastids are developed rather sparingly in this species, and arise in the same manner as that described for *Mucor genevensis* (fig. 21). They persist through the resting period of the zygote and disappear shortly before germination. The zygospores enter the dormant period at the end of about eight days when the hyaline endospore becomes fully differentiated. Reduction division has presumably occurred just prior to this stage, for in zygo-

spores 10–15 days old approximately 40–50 small, apparently unfused nuclei are present. These soon change from the expanded to the unexpanded phase and persist in this condition until the time of germination (fig. 22).

As far as can be determined the germination process has not been previously observed in *Z. dangeardi.* The zygospores are capable of germination after a resting period of about 40 days and retain their viability for at least six months. At the expiration of the rest period the cytoplasm becomes very foamy, the oil plastids disappear and the nuclei resume the expanded condition and undergo mitosis. Shortly the zygospore walls are ruptured and one or more branching germ tubes are put forth (fig. 23). These germ tubes, like those of *Blakeslea trispora,* develop an extensive nutritive mycelium before any sporangiophores are produced. All attempts to induce these germ tubes to develop directly into sporangiophores have failed. The spores of the germ sporangium are all homothallic.

Development of the sporangiophore and sporangium in this genus is in almost all respects similar to that described in *Mucor genevensis* (figs. 24–26). The number of nuclei per sporangiospore is smaller (fig. 27), usually 3–5 nuclei being incorporated in each spore. In some cases the sporangial membrane becomes very finely echinulate with spicule-like projections of calcium oxalate arising from its outer surface. Occasionally a sporangium was observed in which segmentation of the cytoplasm into individual spores had not taken place, and the sporangium had matured with the cytoplasm remaining as a single unit. The columella of this type of sporangium usually is not developed, in which case the resulting structure resembled an azgyospore save that the exospore and suspensor were not well developed. This phenomenon illustrates the probable homology of the sexual and asexual generations, and possibly indicates the direction in which the evolution of the asexual sporangium from the sexual zygospore proceeded. Chlamydospores, although not frequent in this species, are developed in the usual manner when present.

Zygorhynchus moelleri Vuill. The development of this species has been investigated at length by Green (9), Moreau (19), Gruber (10), Atkinson (1), Ling Young (17) and others. Moreau points out that in *Z. moelleri* reduction in the number of fusion nuclei is not so pronounced as in *Z. dangeardi,* and he feels that reduction division occurs at the time of zygospore germination although he was not able to demonstrate this process. Ling Young's (17) results are in accord with those of Moreau. Green (9) was unable to complete a satisfactory cytological study of nuclear behavior in the zygospore because of the small size of the nuclei.

In the strain of *Z. moelleri* used here, there were usually more fusion nuclei present in proportion to the size of the zygospore than were observed

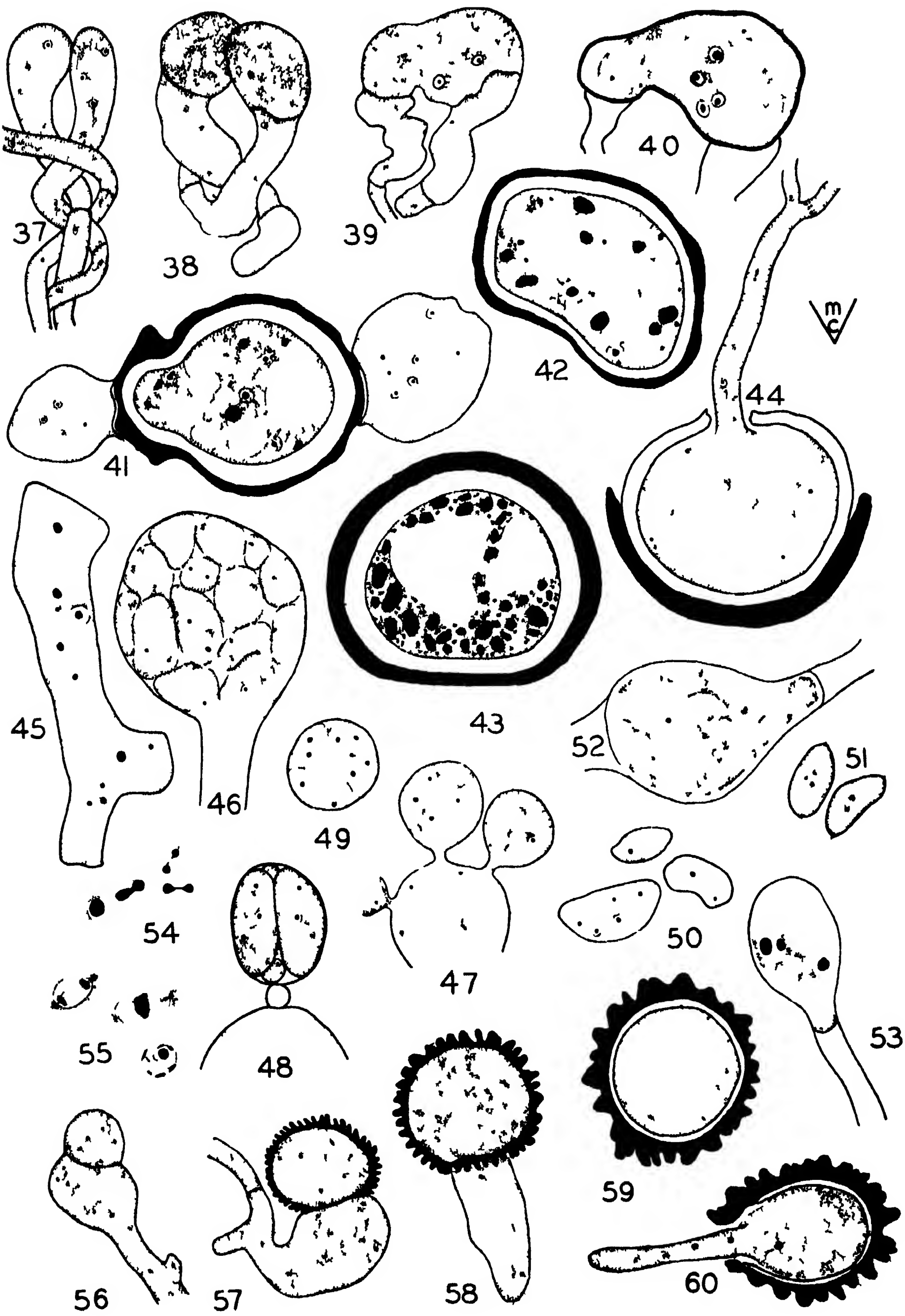
at corresponding stages in *Z. dangeardi,* but the difference is not consistent and certainly does not warrant specific distinction. The remaining stages in the life history were similar in all details to those already described in *Z. dangeardi.* As far as could be determined from nuclear number and volume, reduction division occurs prior to the dormant period, and no evidence was encountered to support Moreau's hypothesis that this process takes place during the germination of the zygote.

ZYGORHYNCHUS VUILLEMINII Namysl. The nuclear behavior in this species has not been previously investigated thoroughly, although Namyslowski (20) has discussed at length the conditions necessary for gametic reproduction. Ling Young (17) in the course of a general discussion of the nuclear behavior in the Mucorales mentions that the nuclear condition in this species is similar to that of *Z. moelleri.* In the material studied here the nuclear condition differed in no appreciable way from that in *Z. moelleri.* Except for minor differences in colony color and zygospore ornamentation there are apparently no reliable criteria for separating them. Since these characters appear somewhat variable the taxonomic validity of the two species may be questioned.

ZYGORHYNCHUS VUILLEMINII var. AGAMUS Namysl. This variety of *Zygorhynchus vuilleminii* is a predominately apandrous strain in which the majority of zygospores develop without the presence of the smaller or male gametangium. In this material only a very occasional zygospore could be

Explanation of figures 37–55, Blakeslea trispora; and of figures 56–60, Zygorhynchus vuilleminii var. agamus

FIG. 37. Progametangial initials in contact (Flemming's; Haematoxylin). ×415. FIG. 38. Gametangia with intervening wall dissolved (Flemming's; Haematoxylin). ×415. FIG. 39. Young coenozygote; nuclei enlarging prior to fusion (Flemming's; Haematoxylin). ×415. FIG. 40. Older coenozygote showing several paired nuclei, and several degenerating nuclei (F.A.A.; Cahal-Brozek). ×415. FIG. 41. Zygospore with fusion nuclei in expanded phase; cytoplasm becoming vacuolate (Chamberlain's; Triple). ×415. FIG. 42. Zygospore 6 days old with fusion nuclei and oil plastids becoming numerous (F.A.A.; Buffer). ×415. FIG. 43. Zygospore in dormant condition; nuclei unexpanded (F.A.A.; Buffer). ×415. FIG. 44. Germinating zygospore (Gilson's; Feulgen). ×415. FIG. 45. Hyphal body with vegetative nuclei in expanded phase (Flemming's; Haematoxylin). ×750. FIG. 46. Sporangium without columella; spores forming (S.M.C.; Feulgen). ×415. FIGS. 47–49. Sporangiola formation (Flemming's; Triple). ×415. FIG. 50. Young sporangiophores; nuclei expanded (F.A.A.; Buffer). ×415. FIG. 51. Mature sporangiospores; nuclei unexpanded (F.A.A.; Buffer). ×415. FIG. 52. Intercalary chlamydospore (F.A.A.; Buffer). ×415. FIG. 53. Young terminal chlamydospore; nuclei expanded; 1 nucleus with 2 central bodies (Flemming's; Haematoxylin). ×600. FIG. 54. Amitotic divisions in columella of sporangiophore, (Flemming's; Feulgen). ×1350. FIG. 55. Mitotic prophase, metaphase and anaphase in hyphae (Flemming's; Triple). ×1350. FIG. 56. Azygospore forming (Chamberlain's; Triple). ×660. FIG. 57. Azygospore 1 day old (Flemming's; Triple). ×660. FIG. 58. Azygospore 7 days old; endospore not yet formed (Chamberlain's; Cahal-Brozek). ×660. FIG. 59. Dormant azygospore (Gilson's; Feulgen). ×660. FIG. 60. Germinating azygospore (Flemming's; Triple). ×660.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found which possessed two authentic suspensors. When undoubted sexual reproduction occurred the nuclear condition followed the same pattern as in *Z. vuilleminii.* In all the studies made on these azygospores the material was carefully examined before fixation and any zygospores were rejected, in order that a more nearly exact conception of nuclear behavior in a typically azygous Mucor might be obtained.

The early stages in the formation of the azygospores closely resembled those encountered in the zygospores. The larger progametangium (fig. 56) grows out from the zygosporic hyphae and quite frequently manifests a weak zygotrophism towards the portion of the zygophore where the smaller or oppositely sexed progametangium would normally be developed. This would indicate that the apandrous condition is a degenerate one and that in certain cases the localization of opposed sexual tendencies has not been completely lost.

The nuclear situation in the larger progametangium is the same as that in the corresponding gametangium of the normal strain. The nuclei through all the developmental stages remain in the expanded phase. When the gametangium has reached its maximum size a heavy, characteristically decorated exospore wall is laid down and the cytoplasm becomes vacuolate, whereupon oil begins to accumulate in the vacuole (figs. 57, 58). The prominent oil plastids seen in the zygotes of other species investigated have not been encountered in the azygospores, although they are developed in the occasional zygospores that are formed on this strain. Inasmuch as no nuclear fusions of any type have been encountered in these azygospores it may be possible that the presence of oil plastids is in some way correlated with the presence of nuclear fusions in the zygospore, since they are apparent in all species where nuclear fusions have been demonstrated, while they occur very rarely in the present variety and in species such as *Sporodinia grandis* where nuclear fusions have not been observed. The fact that these plastids were observed in conjunction with the meiotic nuclei in *Absidia spinosa* is also significant in this connection.

The azygospore enters the dormant period with the appearance of the hyaline endospore when about eight days old (fig. 59). The resting period lasts approximately 30 days. Germination takes place in the same manner described for the zygospores of *Z. dangeardi* (fig. 60). Since no nuclear fusion has ever been observed in this form and since, presumably, nuclei of only one sex are present in the azygospore, germination appears to be almost certainly apomictic. As pointed out above, the infrequent presence of a true zygospore in this strain; the fact that all intergrades between typical sexual reproduction and the apomictic development of the azygospores can be demonstrated; and Namyslowski's report of an occasional culture of this strain in which gametic reproduction is completely suppressed; all point to

the conclusions that in this case the absence of a true sexual reaction is an advanced condition, and that the presence of the sexual reaction is, in *Zygorhynchus* at least, the primitive condition. Attempts have been made to induce a sexual response between this apandrous strain of *Z. vuilleminii* and sexually potent strains of heterothallic species such as *Mucor hiemalis* with very indifferent success. All the evidence indicates that this strain is practically impotent. This might be further interpreted as proof of the contention that, in the Mucorales, heterothallism is the primitive condition, and the assumption of homothallism, which may ultimately be followed by the development of apomictic and finally sexually sterile races, represents an advanced condition.

ABSIDIA SPINOSA Lendner. This homothallic species has been investigated cytologically only by Moreau (19) and Ling Young (17). Moreau reports the nuclear condition as similar to that described by him in *Mucor genevensis*, and Ling Young corroborates him.

The zygospores in this material were very markedly heterogamic, and upon the larger of the two suspensors several long, circinately curved appendages are produced. As the progametangia develop, they push the zygosporic filaments apart, and very shortly after the initial contact is established the suspensors are delimited from the progametangia by transverse walls. These walls in development simulate a closing diaphragm and are formed by a circular cleavage furrow which develops inward from the periphery of the progametangial cell. Since the progametangia are minute at the time when the suspensors are separated from them, it seems probable that these separating walls do not close until a later stage in development. Shortly after the suspensors are delimited, and before the formation of the coenozygote, the appendages of the larger suspensor arise as buds upon its surface. These buds receive nuclei from the suspensors and grow very rapidly, ultimately attaining a length several times that of the zygospore, which is enclosed within these structures. Mitoses of the usual type take place within the developing appendages, but as they reach maturity and cytoplasmic activity ceases amitotic divisions become evident. The appendage walls become much thickened but an open connection is apparently always maintained with the interior of the suspensor (fig. 28). In mature appendages the cytoplasm appears to be evacuated through this opening, back into the suspensor leaving only the rigid appendage wall present. Regeneration experiments, in which the appendages were dissected from the suspensors and isolated on nutrient media, indicate that these appendages are not capable of renewed growth after the heavy wall has been deposited, thus suggesting that the cytoplasm has degenerated or been completely removed. This may be further indication that amitotic nuclear divisions are characteristic of degenerating cytoplasm.

As the cytoplasm of the gametangia mingles to form the coenozygote, the nuclei present in the fusion cell enlarge in size, but no further nuclear divisions are evident; hence the number of nuclei in the coenozygote is not large in comparison with such a form as *Rhizopus nigricans*, in which very rapid nuclear divisions take place at this stage. These unfused nuclei remain in this slightly enlarged expanded state until the exospore wall has been deposited (fig. 28), but a marked association of the nuclei in pairs such as is evident in *Blakeslea trispora* (fig. 40) has not been seen. Soon after the deposition of the exospore wall, which generally occurs in coenozygotes 15–20 hours old, nuclear fusion takes place and as far as could be determined, all the nuclei present in the cell fuse in pairs. No evidence whatever has been seen of the degeneration of unfused nuclei. The fusion nuclei persist in the expanded state until the zygospore is about six days old (fig. 30), during which time vacuoles and oil plastids begin to make their appearance in the cytoplasm.

The best evidence of the nature of the meiotic divisions in the Mucorales has been obtained from studies of division figures in this species. In zygospores 6 to 7 days old nuclear figures which show all the characteristics of meiotic prophases and metaphases have been observed. Figures 29, 63 illustrate a zygospore in which a metaphase and three prophases are visible. Figures 32–35 illustrate the individual stages. It will be noted that these meiotic nuclei are many times the size of the usual vegetative nuclei and that the chromosomes are clearly visible. Since the chromosomes are so markedly enlarged beyond the size of the vegetative nucleus, and since in the nuclei shown in figures 32–35 no indication of any achromatic spindle could be discerned, these figures have been tentatively identified as late diakinesis stages. The large nucleolus-like structure present in these figures proves particularly difficult to interpret. The similarity of these structures to the developing oil plastids scattered through the zygospore at this stage raises grave doubts of the possible interpretation of them as nucleoli. As yet no fundamental difference between these structures and the young oil plastids have been demonstrated. The arrangement of the chromosomes in a plate suggests that figure 35 is a prometaphase in which the spindle has not been fully developed, while the lack of a nucleolus-like body in this figure might give further support to this contention. However, as pointed out above, too much significance cannot at present be attached to the presence or absence within the nuclear vacuole of these putative nucleoli. In the light of present evidence they appear to be oil plastids which have arisen within the nucleus. Their presence in these figures and their similarity to the other oil plastids in the cell gives added credence to Baird's contention (2) that these structures which, in *Phycomyces blakesleeanus*, he terms "reserve bodies," arise within the nuclei and are filled with stainable substances elaborated by the

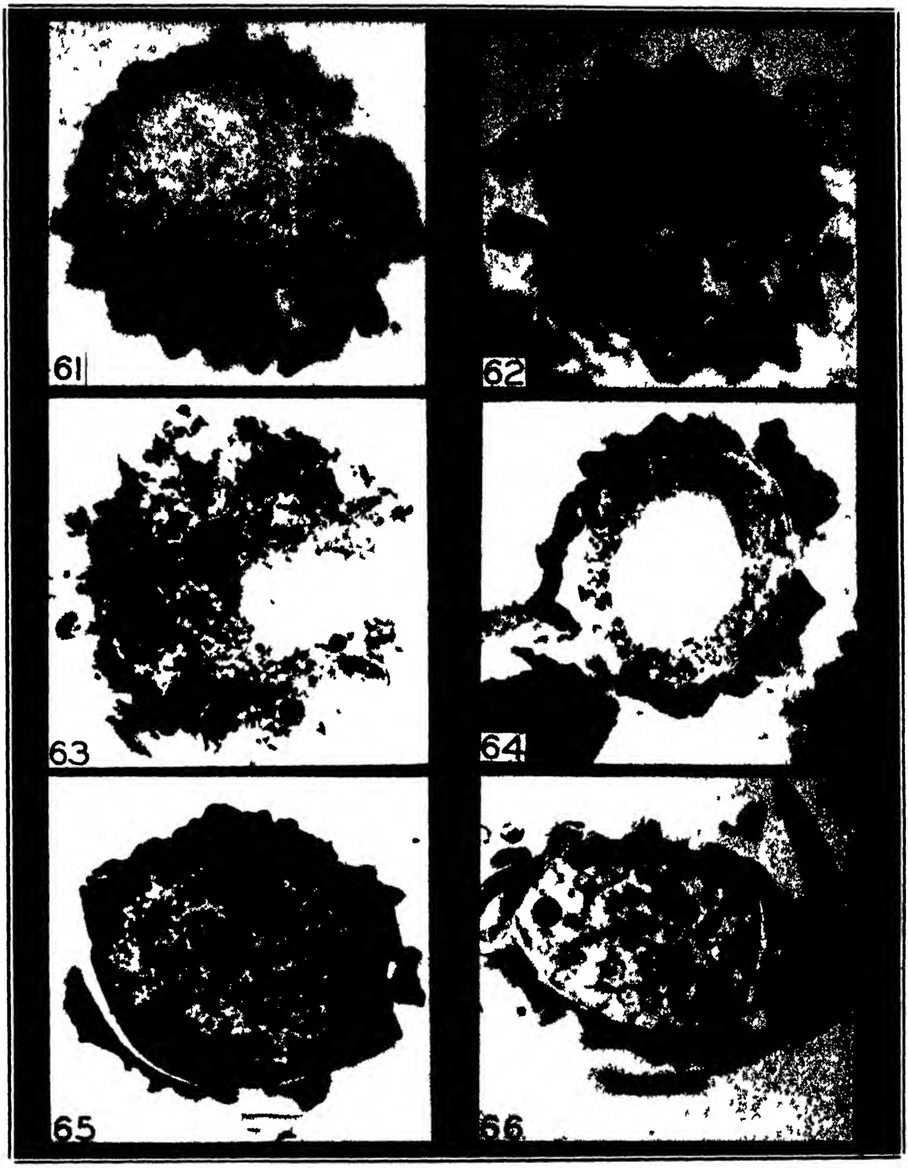

FIG. 61. *Mucor genevensis.* Unfused nuclei in zygospore 3 days old (Gilson's; Haematoxylin). × 700. FIG. 62. *Mucor genevensis.* Fusion nuclei in zygospore 6 days old (Flemming's; Triple). × 700. FIG. 63. *Absidia spinosa.* Prophases of first meiotic division in zygospore 7 days old; oil plastids beginning to accumulate (Craf; Haematoxylin). × 700. FIG. 64. *Absidia spinosa.* Dormant zygospore; nuclei in unexpanded phase (S.M.C.; Feulgen). × 700. FIG. 65. *Absidia spinosa.* Developing oil plastids in zygospore 9 days old; the cloudy appearance is caused by numerous mitochondria scattered in the cytoplasm (Zirkle's; Haematoxylin). × 700. FIG. 66. *Absidia spinosa.* Oil plastids in zygospore 12 days old; the chromosome-like bodies in the plastids are probably fixation artifacts (Craf; Buffer). × 700.

latter. If this is the correct interpretation and these structures merely represent young oil plastids, then the meiotic nuclei must be considered as anucleolate. Further investigation will be necessary to evaluate this supposition.

The chromosome number in all figures where it could be determined was consistently 12. Inasmuch as these figures have been interpreted as prophases of the first meiotic division the somatic chromosome number in *Absidia spinosa* is probably 12. This is significant in the light of Burgeff's (4) remarks concerning the chromosome number in *Phycomyces blakesleeanus.* In division figures which he interpreted as anaphases of the first meiotic division he estimated 24 chromosomes to be present. It is a point of interest that these two species should correspond in their chromosome numbers.

Subsequent stages in the meiotic division have as yet not been clearly demonstrated. Shortly after the stage in zygospore development, where these division figures were found, has been reached, the large fusion nuclei disappear, and about twice the number of smaller apparently haploid nuclei are present (fig. 64). This is the same situation which has been found in the species discussed above and in *Blakeslea trispora,* where as the zygospore enters the dormant period apparently unfused, unexpanded nuclei make their appearance. From these circumstances it has been inferred that the meiotic divisions occur very rapidly just prior to the time when the zygospore enters the dormant condition. At this stage the oil plastids become very numerous and tend to obscure most of the cytological details (figs. 65, 66). The subsequent development and germination of the zygospore of *A. spinosa* differs in no essential from that already described in *Mucor genevensis.*

Germination of the zygospore takes place after a dormant period of 3–4 months. The germ tube takes the form of an arching stolon upon which a single, or occasionally several fasicles, of sporangia are born. This primary stolon will, if placed upon a nutrient medium, develop rhizoids at its point of contact with the substratum, and thus build up an extensive mycelium before the spores in the germ sporangia are shed. The sporangiospores produced in these germ sporangia are all homothallic. The development of the asexual sporangia is similar to that described in the other forms studied here, save that the initial cytoplasmic segmentation is carried even further, so that many uninucleate sporangiospores are produced. In fact in this strain of *A. spinosa* uninucleated spores appear to be the rule rather than the exception, as they are in most other mucors. It will be noted that the nuclear behavior in *A. spinosa* varies in many respects from that which will be reported in a subsequent paper for *Absidia glauca* and *Tieghemella coerulea,* two species previously considered very closely related to *A. spinosa.*

BLAKESLEA TRISPORA Thaxter. Thaxter (28) has described the develop-

ment of the asexual fructifications of this species, and placed it in the Choanephoraceae. Weber and Wolf (29) have demonstrated the heterothallic nature of the species, and discussed the formation of the zygosporic stage. No cytological investigations have been carried out on this form although Wolf (30) has made a partial study of the nuclear condition in the closely related *Choanephora cucurbitarum.*

The nucleus of *Blakeslea* is larger and more conspicuous than in any of the other Mucorales included in this study. In the expanded condition it may reach in some stages a diameter of 4 microns (fig. 53). The number of nuclei in the thallus is fewer than in the less highly specialized species of this order, which probably indicates an advance towards the condition encountered in the higher fungi. In mitosis the achromatic spindle is well developed (fig. 55), but individual chromosomes have not been distinguished.

The formation of the sporangium takes place in much the same manner described by Harper (11) for *Sporodinia,* except that a columella is not regularly formed. The tip of a sporangiophore swells, assuming a more or less spherical shape. The cytoplasm of this sporangial fundament becomes very dense and many nuclei in the expanded condition accumulate within it. Most of these nuclei arise by mitotic divisions from nuclei already present in the fundament. No particular zonation of the nuclei such as is common in the sporangial fundament of most mucors has been observed here. When the sporangium has assumed its maximum size, numerous narrow vacuoles cut the protoplasm into polyhedral multinucleate segments. The vacuoles then become filled with a substance having a glassy, homogeneous appearance. Shortly thereafter the multinucleate segments begin to round up and develop a heavy, dark, longitudinally striate wall (fig. 46). At the poles of each spore a group of fine hair-like appendages radiate outwards from a small cap-like mass on the surface of the spore (fig. 51). These appendages could only be distinguished in mature spores freed from the sporangial wall and could never be demonstrated upon immature spore initials within the sporangium. Consequently their origin remains obscure, although Thaxter (28) feels that they are formed from the intersporal substance.

When a columella is developed in the sporangium, it is cut out by vacuoles at the same time that the spore initials are formed. There was no indication in any of the material studied that a sterile region was present on the site of the columella prior to the formation of the columella wall. This is a more primitive condition than that in *Mucor genevensis* and approaches the type seen in *Mortierella* where no columella is formed. No septum is formed between sporangiophore and columella (fig. 46).

The formation of the sporangiola presents several interesting features. The sporangiophore, instead of producing a single terminal swelling as in sporangium formation, develops instead numerous curiously constricted

dichotomous branches. Septa do not develop at the constrictions. The ultimate dichotomies develop terminal swellings, into which a few nuclei are carried by cytoplasmic streaming. These nuclei undergo several mitotic divisions and come to occupy a peripheral position in the swollen primary head. The center of this head becomes highly vacuolate, and is apparently entirely devoid of nuclei. Upon its surface numerous small swellings arise and enlarge rapidly to form the fundaments of the sporangiola.

The base of the sporangiolum fundament is very narrow and forms a short but distinct stalk. The nuclei of the primary head migrate through this basal stalk into the developing sporangiolum. As far as could be determined no nuclear division takes place within the sporangiolum, the entire nuclear complement of this structure being derived from the primary head. As the sporangiolum approaches its maximum development, longitudinally disposed vacuoles appear which soon separate the cytoplasm into several multinucleate segments (fig. 47). The number of segments is usually three, but as Thaxter (28) points out, a secondary cleavage may result in more than this number of spores being formed. Spore formation after this point differs in no appreciable way from that in the sporangium. As the spores are developing, the basal stalk of the sporangiolum loses its cytoplasmic contents and forms an almost spherical vesicle which functions as a sterigma (fig. 48).

As Thaxter and Weber and Wolf have indicated, all stages of intergradation between sporangia and sporangiola may be found in a single culture of this species. It is clear that these structures are quite homologous and that the sporangiolum represents an extreme modification of the sporangium. The homologies of the curious hyaline, deciduous vesicle formed from the basal stalk of the sporangiolum are not so easily interpreted, but it probably represents a highly modified prototype of the simple sterigma present in *Choanephora.*

The progametangial initials are multinucleate and occasional mitotic divisions are seen in them. As the progametangia are delimited from the intertwined hyphae at the point of contact of the oppositely sexed mycelia, they become broadly clavate or somewhat lobulate and their nuclei are increased by further mitosis. They become somewhat flattened where their tips are in contact (fig. 37) and, after a short period where both progametangia are enlarging rapidly, the wall between them is dissolved; at approximately the same time the suspensors are delimited from the zygosphoric hyphae and from the gametangia by transverse septa (fig. 38). As the wall which separates the gametangia is broken down, the contents of the two cells undergo plasmogamy and continued growth of the fusion cell proceeds. The nuclei in the young coenozygote divide mitotically several times, and there come to be approximately 10–20 nuclei in the cell. About 6 hours after the formation of the coenozygote most of the nuclei enlarge considerably and

approach each other, remaining for some hours associated in pairs (figs. 39, 40). As the exospore wall appears, about 15 hours after the formation of the coenozygote, these paired nuclei fuse. The fusion nuclei remain in the expanded phase and at this stage are the most conspicuous bodies in the young zygospore. Very shortly after fusion takes place the cytoplasm becomes vacuolate and oil plastids make their appearance and lie scattered at random in the cell (fig. 41). These oil plastids contain a bright orange oily substance which imparts the characteristic color to the zygospores. The fusion nuclei remain in the expanded state until the zygospore is at least 6 days old (fig. 42). During this period the oil plastids tend to group around the central vacuoles in the cell, so that when viewed externally the spore appears to contain a single oil globule.

Between the sixth and tenth day after the formation of the coenozygote the expanded fusion nuclei disappear and smaller apparently unfused nuclei become evident. These smaller nuclei are several times as numerous as the fusion nuclei. Although the actual divisions were not seen, it is thought probable that meiosis occurred at this point and that these smaller nuclei are haploid. At this point the abundance of oil plastids in the cell renders the interpretation of nuclear structures somewhat hazardous. Shortly after this point the haploid nuclei assume the unexpanded phase, the oil plastids accumulate densely in the cytoplasm and the zygospore enters the dormant condition (fig. 43). No further changes ensue until germination. The behavior of the mitochondrial elements during the formation and development of the coenozygote follows the course described for the preceding species, and show the same connection with the oil plastids.

Germination of the zygospores takes place in the third month after their formation, and is identical with that seen in the species of *Zygorhynchus* studied (fig. 44). The germ tube does not form a sporangium directly, but produces instead an extensive submerged vegetative mycelium which ultimately bears sporangiophores. Repeated attempts have been made to induce the germ tube to develop directly into a sporangiophore by isolating germinating zygospores upon non-nutrient and solid substrates, but all the germ tubes aborted and no further growth ensued. It seems possible that the limited amount of cytoplasm present in these small zygospores may prohibit the formation of a complex sporangiophore. Segregation of sex is apparently complete at meiosis, all the spores of any given sporangium giving rise to mycelium of only one sex.

DISCUSSION

A consideration of the nuclear behavior throughout the life cycle of the 8 species studied here indicates that except for minor differences in size and number of nuclei the same basic behavior-pattern is common to all of them. The salient features of this pattern are as follows: the haploid condition is

maintained throughout the development of the thallus; karyogamy occurs during the development of the coenozygote usually as the exospore appears, and all the nuclei present in the coenozygote fuse in pairs; the number of the nuclei in the coenozygote is not large, rarely exceeding 100; at the time of syngamy the nuclei enlarge considerably; no degeneration of unfused nuclei is evident; reduction division follows karyogamy closely and takes place before the zygote enters the dormant condition; the dormant period persists from 1–6 months, depending upon the species; at the time of germination either a single sporangiophore or an extensive submerged germ mycelium is produced by the zygospore; the spores produced in the germ sporangia are all of the same sex, indicating that sex segregation has been complete at the time of meiosis. This type of nuclear behavior in the zygote is correlated with a general similarity in the behavior of the nuclei during sporangium and sporangiospore formation. A progressive specialization can be noted in the amount of cytoplasmic segmentation in the sporangium, during spore formation, from the condition observed in certain cases in *Zygorhynchus* where no cytoplasmic segmentation occurs to that in *Absidia spinosa* where ultimately uninucleate portions of cytoplasm are cut out. In the zygospore a somewhat similar situation may be noted in the gradual reduction in the number of nuclei incorporated in the coenozygote from the condition in *Mucor genevensis* where many nuclei are present towards that in *Blakeslea trispora* where only a few nuclei enter the developing coenozygote. This reduction in nuclear number seems to be associated with an increase in nuclear size. It is possible that when further investigations have been carried out on intermediate forms valuable clues to the phylogenetic development of this group may be obtained from a comparison of nuclear behavior in different species. However, with the meagre evidence available at present any such phylogenetic speculation seems unwarranted.

The foregoing study has revealed several points which require further investigation before a satisfactory explanation can be offered. Sex segregation in some of the heterothallic forms, as outlined under the description of *Mucor hiemalis,* appears quite anomalous. It is probable that due to the small size of the nucleus and the obvious impossibility of tracing an individual nucleus through the life cycle, a satisfactory answer will only be obtained through genetical analysis of the progeny of many zygospore germinations. The relationship of the elements of the chondriome to the elaboration of the reserve substances in the zygospores, and other resistant structures and in particular the relation of the mitochondria to the developing oil plastids needs further study. The possible connection between the oil plastids and the syngamic nuclei must also be considered. Further investigations along these lines are contemplated. A subsequent paper in this series will outline the nuclear behavior in 6 additional species of the Mucorales.

SUMMARY

1. By the use of several techniques not previously applied to this group the nuclear behavior in 9 species of the Mucorales is traced through all the stages in the life history.

2. In these species, with the exception of minor differences in nuclear number and size, the same basic behavior is observed at all stages. This will be referred to as the "*Mucor* pattern" of nuclear behavior.

3. In the "*Mucor* pattern" all functional zygospore nuclei undergo karyogamy followed by immediate reduction division prior to the onset of dormancy in the zygospore. Segregation of sex is complete in the heterothallic species at meiosis.

4. In an azygous variety, *Zygorhynchus vuilleminii* var. *agamus,* no nuclear fusion or reduction is encountered in the azygospore which, in this case, cannot be regarded as a true sexual spore.

5. The relationship of the chondriome to the deposition of reserve substances is discussed and the possible connection between the mitochondria and the developing oil plastids is suggested.

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VITAMIN DEFICIENCIES OF TRICHOPHYTON DISCOIDES

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Mackinnon (8) reported that strains of *Actinomyces* stimulated the growth of *Trichophyton discoides* on a glucose-peptone agar. He found that the addition of thiamine to the agar medium had the same effect as *Actinomyces* on the growth of *Trichophyton*, and called attention to the considerable variation in the character of colony growth produced by the addition of thiamine to a glucose-peptone agar. From his results it appears that *T. discoides* suffers from a thiamine deficiency which is not satisfied by the thiamine in peptone[1] in the amount used, but is by thiamine synthesized by *Actinomyces albus*.

The present paper is a further report on the nutritional requirements of *Trichophyton discoides* Sabouraud, with special reference to its vitamin deficiencies. Part of the experimental work was performed at the Institute of Hygiene in Montevideo, Uruguay, and the balance at the New York Botanical Garden.

MATERIALS AND METHODS

The strain of *T. discoides* originally employed by Mackinnon was used in all experiments. Unless otherwise indicated the organism was grown at 35° C. on agar slopes in pyrex test tubes, 20×150 mm. Each tube contained 8 ml. of a basal medium composed per liter of 50 g. dextrose, 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4 \cdot 7H_2O$, 2.0 g. asparagine, and 15 g. purified agar. To this medium the following trace elements in p.p.m. were added: 0.005 B, 0.02 Cu, 0.1 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo, and 0.09 Zn. The dextrose was Corn Products C.P. The asparagine was purified by treatment with Norit A and recrystallization from redistilled alcohol. The agar was purified by extraction with aqueous pyridine and alcohol (14). The stock cultures were maintained on the basal medium to which 10 mμ moles of thiamine and 100 mg. of neopeptone were added to each tube. Inoculations were made by transferring from a stock culture a bit of mycelium about the size of a pinhead.

In estimating the effects of various media on the growth of *Trichophyton* observations were made at intervals on the character of the growth and colony diameters were measured.

EXPERIMENTS

Relation to Thiamine and its Intermediates. *T. discoides* was found

[1] Robbins and Schmidt (12) found peptone to have a small amount of thiamine or its intermediates.

to require molecular thiamine. Its thiamine deficiency could not be satisfied by pyrimidine,[2] by thiazole or by a mixture of the two intermediates.

This was determined by testing the effect of thiamine and its intermediates on the growth of the organism on a dextrose-peptone medium. Although bacto-peptone contains little thiamine, the small amount present was destroyed by heating the peptone under alkaline conditions. A 33.3 per cent solution of peptone was heated at pH 10.0 for 20 minutes at 135° C. The reaction was then adjusted to pH 7.0. Tests with *Phytophthora cinnamomi* demonstrated that this treatment destroyed all, or very nearly all, the thiamine originally present in the peptone. A medium was prepared containing per liter 18 g. agar, 40 g. dextrose, and 30 ml. of the "heated peptone" solution. Fifty ml. of this medium or the same medium supplemented with thiamine or its intermediates were placed in flasks with a flat side 5 cm. by 14 cm. After sterilization the media were inoculated with *T. discoides*. The experiment was performed in triplicate and the results are given in table 1. Good growth was obtained only in those flasks to which thiamine was added; in fact pyrimidine at the amounts used appeared to be somewhat toxic.

TABLE 1. *Effect of thiamine and its intermediates on growth of Trichophyton discoides on a dextrose-agar medium containing peptone heated under alkaline conditions to destroy thiamine*

Additions to basal medium containing "heated peptone"	Development of Trichophyton
None	Scanty glabrous growth.
100 or 1000 mμ moles thiamine	Velvety colonies measuring 15 to 20 cm. in diameter after 15 days.
100 or 1000 mμ moles thiazole	Scanty glabrous growth.
100 or 1000 mμ moles pyrimidine	No growth.
100 mμ moles of thiazole and of pyrimidine or 1000 mμ moles each	No growth.

In a second experiment 20 ml. of the medium were used in Erlenmeyer flasks. Thiamine or its intermediates were added in amounts of 1, 5, and 20 mμ moles per flask. Abundant growth was obtained in those flasks containing thiamine; the development was somewhat greater with 5 and 20 mμ moles than with 1 mμ mole. Growth in the medium with no supplements was scanty; it was scanty also in the media containing the various amounts of thiazole, in those with 1 mμ mole or 5 mμ moles of pyrimidine, and in those with 1 or 5 mμ moles of both intermediates. No growth was observed in the flasks containing 20 mμ moles of pyrimidine, again indicating injury from the pyrimidine. Other experiments seemed to prove that the inhibitory effect of the pyrimidine was reduced or eliminated in media containing sufficient thiamine. Study of the apparent antagonism between pyrimidine and thiamine was not pursued further.

[2] The terms pyrimidine and thiazole are used in this paper to refer to 2-methyl-5-bromo-methyl-6-amino-pyrimidine and 4-methyl-5-β-hydroxyethyl-thiazole respectively.

Relation to Vitamins other than Thiamine. Although *T. discoides* grew quite satisfactorily on a mineral-dextrose-peptone agar supplemented with thiamine, it did not grow on the same medium with peptone replaced by minerals and asparagine. Evidently peptone supplied something essential for the growth of the organism not included in the minerals and asparagine by which it was replaced. Peptone is a complex mixture, containing minerals, amino acids, and several vitamins as well as other types of organic compounds. Since asparagine is a good source of nitrogen for most fungi and the basal medium contained all the minerals recognized as necessary, our attention was naturally directed toward the effect of vitamins other than thiamine.

A preliminary experiment showed that the addition to the basal medium of a mixture of eleven vitamins or vitamin-like substances allowed *T. discoides* to grow (table 2). The vitamins were added to each tube in the following amounts: pimelic acid 10 μg., para-aminobenzoic acid 10 μg., 2-methyl-1,4-naphtho-hydroquinone diacetate 5 μg., calcium pantothenate 5 μg., lactoflavin 10 mμ moles, nicotinamide 5 mμ moles, thiamine 5 mμ moles, pyridoxine 5 mμ moles, guanine 5 mμ moles, hypoxanthine 5 mμ moles, and *i*-inosotol 1 mg. The colonies produced in the presence of the vitamins were white, velvety, raised several mm., convoluted and with sharp well-defined edges. They were not so extensive as those which developed on a thiamine-peptone agar where the growth was flatter, less convoluted, and in our experiments somewhat gray brown in color.

TABLE 2. *Effect of various supplements on growth of T. discoides on agar medium containing minerals, dextrose, asparagine and thiamine*
See text for composition of vitamin mixture and amino acids.

Additions to 8 ml. of basal agar medium containing 10 mμ moles thiamine	Average colony diameter in mm. after	
	13 days	18 days
None	0.0	0.0
Vitamin mixture	7.0	7.5
0.1 ml. D_R fraction	0.0	0.0
Vitamin mixture and 21 amino acids	7.0	7.5
Vitamin mixture, 21 amino acids and 0.1 ml. D_R fraction	10.0	13.0
Vitamin mixture and D_R fraction	10.0	13.0
Peptone 100 mg.	16.0	27.0

Relation to Thiamine, Pyridoxine, and I-Inositol. To determine which of the eleven vitamins favorably affected the growth of *T. discoides* the organism was grown on the basal medium plus ten of the vitamins, omitting one after another of the eleven in turn. For example, one set of tubes contained all but pimelic acid, another all but para-aminobenzoic acid, a third all but calcium pantothenate and so on. It was found that no growth occurred when thiamine, pyridoxine, or *i*-inositol was omitted, even though the

other ten vitamins were present. Omission of any one of the other seven had little effect on the growth of the organism.

It appeared, therefore, that *T. discoides* suffered from a complete deficiency for thiamine, pyridoxine and *i*-inositol. This was confirmed by cultivating the organism on the basal medium supplemented per tube with 5 mμ moles thiamine, 5 mμ moles pyridoxine, and 1 mg. *i*-inositol singly and in all possible combinations (table 3). At the same time tubes were pre-

TABLE 3. *Effect of thiamine, pyridoxine, i-inositol and other supplements on growth of T. discoides on agar medium containing dextrose, minerals and asparagine*
See text for vitamin mixture.

Additions to 8 ml. of basal medium	Average colony diameter in mm. after		
	12 days	23 days	30 days
5 mμ moles thiamine	0.0	0.0	0.0
5 mμ moles pyridoxine	0.0	0.0	0.0
1 mg. *i*-inositol	0.0	0.0	0.0
thiamine and pyridoxine	0.0	0.0	0.0
thiamine and inositol	0.0	0.0	0.0
pyridoxine and inositol	4.5 (thin)	5.0 (thin)	5.0 (thin)
thiamine, pyridoxine and inositol	6.0	8.5	14.0
vitamin mixture	6.0	10.0	11.0
thiamine and 100 mg. peptone	22.0	30.0	31.0
thiamine, peptone and inositol	23.0	32.0	32.0
thiamine, pyridoxine and calcium phytate	4.0 (flat)	7.5 (flat)	9.0 (flat)

pared containing the basal medium plus the mixture of eleven vitamins in the amounts previously given and the basal medium plus 5 mμ moles of thiamine and 100 mg. peptone per tube. No growth was observed even after more than 30 days in the tubes supplemented with thiamine, with pyridoxine, with *i*-inositol, with thiamine and pyridoxine, or with thiamine and *i*-inositol. A thin, flat, waxy colony developed in the tubes containing the basal medium plus pyridoxine and inositol which increased little in size after the 12th day of incubation. Sub-cultures from these colonies made at the end of 34 days failed to grow on the basal medium supplemented with pyridoxine and *i*-inositol but grew normally on the medium supplemented with thiamine, pyridoxine, and *i*-inositol. We concluded that the small amount of growth on the medium supplemented with pyridoxine and *i*-inositol was the result of a minute amount of thiamine carried over with the inoculum.

Growth on the basal medium plus all three vitamins was quite satisfactory (fig. 1), and about the same as on the medium supplemented with the eleven vitamins. However, growth was much more rapid (table 3) on the basal medium to which thiamine and peptone were added. This experiment confirmed the conclusion that *T. discoides* has complete deficiencies for thiamine,

pyridoxine and *i*-inositol. Unless all three of these substances were present in the medium no growth occurred.

Since the fungus grew on the basal medium supplemented with thiamine and peptone, it follows that peptone in the amount used supplied sufficient pyridoxine and *i*-inositol or physiologically equivalent substances to satisfy the needs of the organism. However, the considerably more rapid growth on the peptone medium as compared with that on the medium supplemented

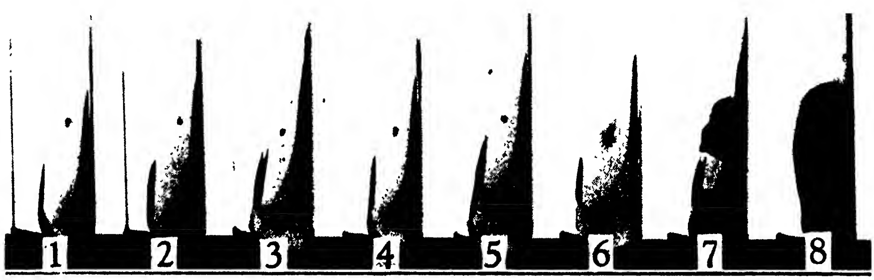

FIG. 1. Growth of *T. discoides* on a basal mineral-dextrose medium containing asparagine and 1.5 per cent purified agar supplemented with (1) thiamine; (2) pyridoxine; (3) *i*-inositol; (4) thiamine and pyridoxine; (5) thiamine and inositol; (6) pyridoxine and inositol; (7) all three vitamins; (8) thiamine and peptone. Age 32 days.

with the three (or eleven) vitamins suggested that peptone contains some unidentified factor or factors important in the development of this organism.

Attempts to Substitute other Vitamin Combinations for Thiamine, Pyridoxine, and I-Inositol. In the course of our investigation a considerable number of vitamins alone or in combination were used as supplements to the basal medium. Growth was obtained with those combinations only which included thiamine, pyridoxine, and *i*-inositol. In addition to the various vitamins tested singly or in combination with negative results and reported earlier in this paper, adding the following to the basal medium did not permit growth; biotin; thiamine and biotin; pyridoxine and biotin; thiamine, pyridoxine, and biotin; thiamine and hypoxanthine; thiamine and a D_R fraction;[3] thiamine, hypoxanthine and a D_R fraction; thiamine and calcium pantothenate; thiamine, biotin and calcium pantothenate; thiamine and nicotinamide; thiamine, nicotinamide, and biotin; thiamine, nicotinamide, calcium pantothenate, and biotin; para-aminobenzoic acid; calcium pantothenate and para-aminobenzoic acid; calcium pantothenate and para-aminobenzoic acid; calcium pantothenate, para-aminobenzoic acid, and nicotinamide; thiamine, calcium pantothenate, para-aminobenzoic acid,

[3] The D_R fraction is the filtrate from an extract of white potato tubers which has been treated with charcoal (13).

nicotinamide and biotin; thiamine and pimelic acid; thiamine and lactoflavin; thiamine and 2-methyl-1,4naphtho-hydroquinone diacetate; thiamine, lactoflavin, *i*-inositol, pimelic acid, and 2-methyl-1,4-naphtho-hydroquinone diacetate; thiamine, pimelic acid, para-aminobenzoic acid, 2-methyl-1,4-naptho-hydroquinone diacetate, calcium pantothenate, lactoflavin, nicotinamide, guanine, hypoxanthine, *i*-inositol, and a D_R fraction.

Relation to Calcium Phytate and an Inositol Phosphatide. Comparisons of growth on the basal medium supplemented with thiamine, pyridoxine, and *i*-inositol, and on the same medium with inositol replaced with an equal weight of calcium phytate or a phosphatide containing inositol[4] were made. To the medium in each tube 5 mμ moles of thiamine, 5 mμ moles of pyridoxine and 1 mg. of inositol, calcium phytate or the phosphatide were added. *Trichophyton* developed slowly on the medium containing calcium phytate; there was little growth during the first week. At the end of 30 days the colony diameter was 9.0 mm. as compared to 14.0 mm. on the medium containing

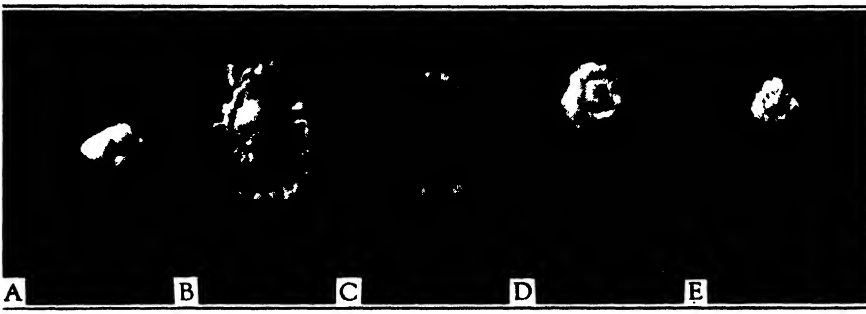

FIG. 2. *T. discoides* grown on a basal mineral-dextrose medium containing asparagine and 1.5 per cent purified agar supplemented with (A) thiamine, pyridoxine and inositol; (B) thiamine and peptone; (C) eleven vitamins and gelatin hydrolysate; (D) thiamine, pyridoxine and a phosphatide; (E) thiamine, pyridoxine and calcium phytate. Age 28 days.

inositol (table 3). The difference in growth was, however, greater than indicated by the colony diameters. On the phytate medium the colony was flat, closely appressed to the agar and with a powdery surface; on the inositol medium it was raised 3 or 4 mm. with a velvety surface. The phosphatide replaced inositol more nearly than did the calcium phytate. Colony diameters on the medium containing the phosphatide were equal to those on the inositol medium, but the colony height on the latter medium was 2 or 3 times that on the phosphatide medium (fig. 2). Neither calcium phytate nor the phosphatide was as satisfactory as inositol.

Effective Quantities of Thiamine, Pyridoxine, and I-Inositol. The effect of the quantity of each of the three vitamins for which *T. discoides*

[4] The phosphatide was kindly supplied by D. W. Wooley and is described in a paper published by Folch and Wooley (2).

shows a complete deficiency was determined by growing the organism on the basal agar medium containing a fixed and adequate amount of two of the vitamins with different quantities of the third. For inositol the medium contained per tube 10 mμ moles of thiamine and 50 mμ moles of pyridoxine with 2, 1, 0.5, 0.1, 0.01, and 0.00 inositol; for pyridoxine it contained 10 mμ moles of thiamine and 1 mg. of inositol with 50, 10, 1, 0.5, 0.01, 0.001, and 0.000 mμ moles pyridoxine; for thiamine we used 10 mμ moles of pyridoxine and 1 mg. of inositol with 10, 5, 1, 0.1, 0.01 and 0.000 mμ moles of thiamine.

At the end of two weeks no difference in the growth with from 2.0 to 0.1 mg. inositol was noticeable, but there was a marked reduction where 0.1 mg. was supplied. At the end of a month the growth with 0.5, 1, and 2 mg. inositol was about the same. There was a slight reduction with 0.1 mg. and a marked reduction with 0.01 mg. per tube; the colonies were flattened and grew closely appressed to the agar. There was little growth in the tubes lacking inositol, mostly under the agar and probably at the expense of inositol carried over with the inoculum (fig. 3).

In the tubes in which pyridoxine varied little difference in growth was noted at the end of two weeks with 50, 10, or 1 mμ mole of pyridoxine. There was a progressive reduction in growth in the tubes with 0.05 and 0.01 mμ mole and little difference between those with 0.001 mμ mole and the check. At the end of 4 weeks the growth in the tubes with 10 mμ moles and 1 mμ mole of pyridoxine was about the same, but in the former the growth was snow white, while in the latter it was somewhat grayish. With 0.05 mμ mole there was a distinct flattening of the colony, with 0.01 mμ mole the colony was reduced in diameter and quite flat, growth with 0.001 mμ mole was only slightly better than the check (fig. 3).

At the end of two weeks little difference was noted in the effects of 10, 5, and 1 mμ mole of thiamine, but with 0.1 and 0.01 mμ mole there was a progressive reduction in growth. At the end of a month there was no difference between the growth in tubes containing 10 and 5 mμ moles of thiamine, but the colonies appeared grayish with 1 mμ mole. With 0.1 mμ mole of thiamine the colonies were flattened with a raised spot in the center and with 0.01 mμ mole the colonies were thin, grayish and closely appressed to the agar. The check showed still less growth (fig. 3).

It appeared from these results that between 0.1 and 0.5 mg. of inositol and between 1 and 10 mμ moles of pyridoxine and thiamine per tube gave maximum growth of *T. discoides* for 4 weeks in our basal medium. These figures would be reduced for shorter periods of growth.

Inositol in Agar, Gelatin, and other Natural Products. The ability of *T. discoides* to grow on a thiamine-peptone agar demonstrated the presence in peptone of both pyridoxine and *i*-inositol or physiologically equivalent substances. One hundred mg. of neo-peptone per tube appeared to supply suf-

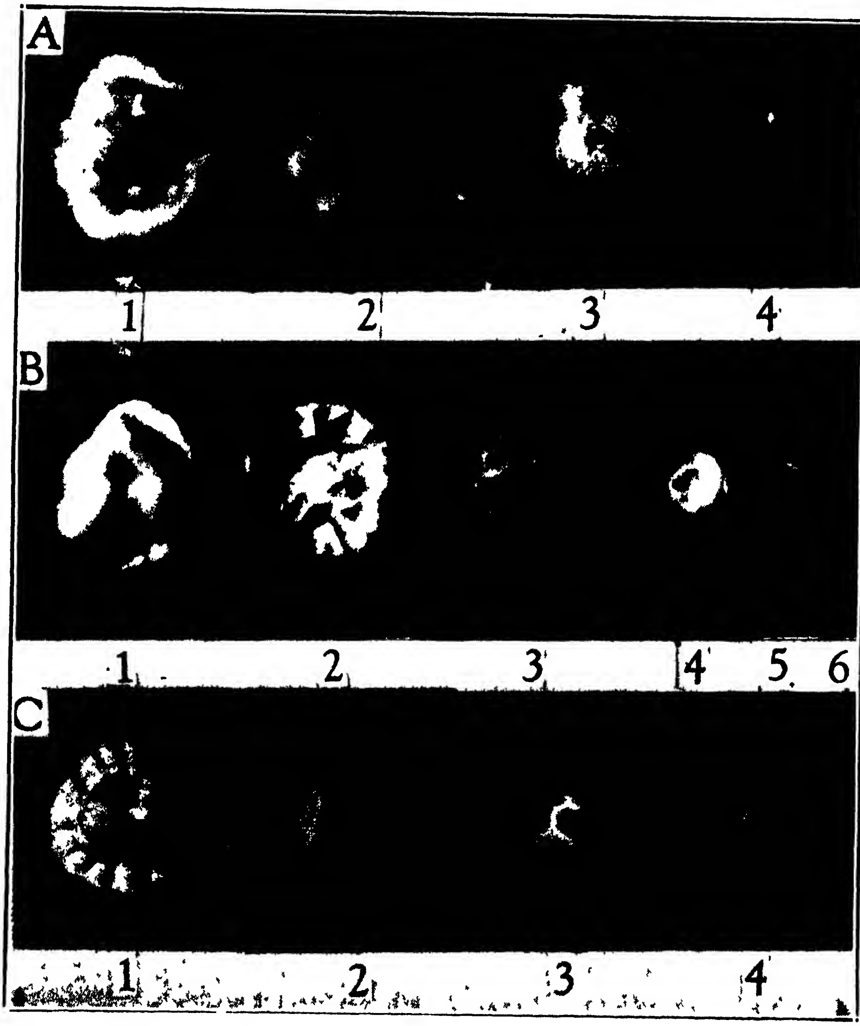

FIG. 3. Amount of vitamins and growth of *Trichophyton discoides* on mineral dextrose medium containing agar and asparagine and supplemented as follows: A. 10mμ moles thiamine, 50 mμ moles pyridoxine and (1) 0.5 mg. *i* inositol; (2) 0.1 mg. *i*-inositol; (3) 0.01 mg. *i* inositol; (4) no inositol. B. 10 mμ moles thiamine, 1 mg. *i* inositol and (1) 10 mμ moles pyridoxine; (2) 1 mμ mole pyridoxine; (3) 0.05 mμ mole pyridoxine; (4) 0.01 mμ mole pyridoxine; (5) 0.001 mμ mole pyridoxine; (6) no pyridoxine. C. 10 mμ moles pyridoxine, 1 mg. *i*-inositol and (1) 1 mμ mole thiamine; (2) 0.1 mμ mole thiamine; (3) 0.01 mμ mole thiamine; (4) no thiamine. Age 4 weeks. About twice natural size.

ficient *i*-inositol for maximum growth since the addition of inositol to the thiamine-peptone medium did not improve growth. One hundred and twenty-five mg. of Difco agar contained less *i*-inositol than required to induce growth of *T. discoides*. We failed to observe any growth on 8 ml. of our basal medium

prepared with unpurified Difco agar and supplemented with thiamine and pyridoxine. The D_R fraction from potato containing 13 mg. of dry matter furnished enough *i*-inositol to permit some growth though considerably less than the maximum.

I-inositol or physiologically equivalent material was found to be present also in hydrolyzed gelatin, hydrolyzed casein, and hydrolyzed egg albumen.[5] This was demonstrated by growing the fungus on the basal medium supplemented per tube with 10 mμ moles of thiamine to which 100 mg. of hydrolyzed gelatin, egg albumen, or casein were added. At the end of 3 weeks colonies with the following diameters had developed: on gelatin, 19 mm.; on the egg albumen, 18.3 mm.; on casein, 25 mm.; on peptone, 34.6 mm. The diameter on the basal medium plus thiamine, pyridoxine, and inositol was 8.3 mm. It is not possible to state the quantity of inositol present in these products from this observation but it was appreciable.

Relation to Unidentified Factors. Attention has been called above to the probability that peptone contains some unidentified factors important in the growth of *T. discoides*. This was suggested by the more rapid growth observed on a medium containing thiamine and peptone than on one containing thiamine, pyridoxine, and *i*-inositol. The unidentified factors were not found among the following substances: biotin, hypoxanthine, guanine, pimelic acid, para-aminobenzoic acid, calcium pantothenate, lactoflavin, nicotinamide, or 2-methyl-1,4-naphtho-hydroquinone diacetate. The addition of these vitamins to the basal medium containing thiamine, pyridoxine and *i*-inositol was of little or no benefit. We failed also to improve the basal medium containing the 12 vitamins listed above by the addition of 21 amino acids.[6] The further addition per tube of 100 mμ moles of choline, adenine, xanthine, cytosine, orotic acid, thymine, and uracil was not found to be beneficial. No benefit was observed by the addition per tube of 20 μg. of a concentrate of folic acid and of 23 amino acids[7] to the basal medium containing the 12 vitamins, 21 amino acids, and the pyrimidine and purine bases listed above.

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[5] Difco gelatin was hydrolyzed by boiling for 24 hours with 8N H_2SO_4. The sulfuric acid was removed by treatment with $Ba(OH)_2$. Difco egg albumen coagulated was hydrolyzed in the same way. The hydrolyzed casein was a product from Mead Johnson and Co. with the laboratory number 92–403.

[6] These amino acids and the amounts added per tube were as follows: 0.15 mg. *d*-arginine, 0.075 mg. *dl*-α-alanine, 0.15 mg. *l*-aspartic acid, 0.15 mg. *d*-glutamic acid, 0.075 mg. glycine, 0.05 mg. β-alanine, 0.02 mg. *dl*-lycine HCl, 0.01 mg. *dl*-norleucine, 0.01 mg. serine, 0.01 mg. *dl*-threonine, 0.01 mg. *dl*-valine, 0.01 mg. *d*-cysteine HCl, 0.02 mg. *l*-histidine HCl, 0.01 mg. *l*-hydroxyproline, 0.01 mg. *dl*-methionine, 0.01 mg. *dl*-phenylalanine, 0.01 mg. *l*-proline, 0.02 mg. *l*-tryptophane, 0.005 mg. tyrosine and 0.005 mg. thyroxine.

[7] The amino acids included 0.1 mg. each of *dl*-α-amino butyric acid, *dl*-β-amino-*n* butyric acid, α-amino isobutyric acid, *dl*-α-amino caproic acid, *dl*-α-amino-*n*-valeric acid, *dl*-δ-amino-*n*-valeric acid, *dl*-aspartic acid, betaine HCl, *dl*-benzoylalanine, creatinine, *d*-cysteine, glutamine, glutathione, *dl*-homoaptine, *m*-nitro hyppuric acid, *p*-nitrophenylglycine, *n*-phenylglycine, pyrazine monocarboxylic acid, pyrazine 2, 3 dicarboxylic acid, sarcosine, spermadine phosphate, and taurine.

In spite of these negative results it may be entirely possible that the unidentified factors are included among the amino acids or other compounds tested. The active compound, if it was one of the 60 substances tested with negative results, may have been used in too small an amount, or its activity may have been masked by the toxicity of another member of the complex.

The unidentified factor or factors appeared to be present in a D_R fraction prepared from potato, in malt extract, in casein hydrolysate, in Difco gelatin both hydrolyzed and unhydrolyzed, and in egg albumen hydrolysate. The addition per tube of a D_R fraction containing 13 mg. of dry matter to the basal medium plus the 12 vitamins was beneficial though not as much so as the addition of an equal amount of neo-peptone. The benefit observed from 100 mg. of Difco desiccated malt extract was about equivalent to that obtained with 13 mg. of the D_R fraction. Difco gelatin and casein hydrolysate were almost as beneficial as peptone when compared on a dry weight basis. Some benefit was obtained with the addition per tube of 4 mg. of peptone or gelatin and perhaps with 1 mg.; 16 mg. doubled the growth as measured by colony diameters, and the effect increased still further with 64 or 128 mg. per tube (table 4). The action of the gelatin, casein, or peptone was not the

TABLE 4. *Effect of various additions to an agar medium containing dextrose, minerals, asparagine and eleven vitamins on the growth of T. discoides.*
The unit for the D_R fraction was 1.3 mg. instead of 1.0 mg.

Additions to 8 ml. of basal medium plus 11 vitamins	Average colony diameter in mm. after 14 days					Average colony diameter in mm. after 24 days				
	Gelatin	Peptone	Asparagine	Casein	D_R fraction	Gelatin	Peptone	Asparagine	Casein	D_R fraction
0 ...	6.0	6.0	6.0	6.0	6.0	11.0	11.0	11.0	11.0	11.0
1 mg.	7.5	4.0	4.0	...	7.5	14.0	11.0	10.0	...	13.0
4 mg.	9.0	4.0	5.0	...		15.0	14.0	11.0	.	.
16 mg.	12.0	13.0	6.0	17.0	11.0	25.0	27.0	10.0	23.0	22.0
64 mg.	15.0	20.0	4.0	18.0		29.0	32.0	8.0	32.0	
128 mg.	15.0	20.0	4.0	...	6.0	35.0	34.0	8.0	...	11.0

result of increasing the nitrogen supply. This followed because increasing the asparagine had no comparable effect. Since gelatin was beneficial the effect of 4 mg. of glycine per tube was tested. No benefit was observed. Further work on the nature of the unidentified factors is in progress.

DISCUSSION

T. discoides is parasitic on calves and produces an infection of man which presents the same clinical characteristics. *T. album* is very similar to *T. discoides* in its pathogenic effects and is distinguished from the latter organ-

ism by differences in the appearance of colonies upon Sabouraud's medium. However, *T. discoides* closely resembles *T. album* when grown on Sabouraud's dextrose medium to which thiamine had been added and an investigation of *T. album* demonstrated that it synthesized thiamine. This suggested that *T. discoides* may be a mutant of *T. album*, a mutation which involved the loss of the ability to synthesize thiamine. These observations emphasize also the importance of the vitamin content of a medium in determining the diagnostic characteristics of an organism which is identified largely by its colonies.

The nutritional requirements of species of *Trichophyton* have received relatively little attention. Mosher, Saunders, Kingery and Williams (9) found that *T. interdigitale* did not grow in a mineral-sugar medium containing amino acids. Addition of crystalline thiamine, *i*-inositol, a highly concentrated preparation of pantothenic acid, or crude lactoflavin permitted growth. Some combinations of these supplements were more effective than the single substances. Oyama (10) reported that thiamine had no effect on the growth of *T. crateriforme* in a solution of minerals, sugar, and asparagine, but a concentrate of rice polishings improved growth. On the other hand the growth of *T. rosaceum* was improved by the addition of thiamine or of a concentrate of rice polishings. Peck and Rosenfeld (11) state that the growth of *T. gypseum* was not affected by vitamins A, B, and D, but that from 0.09 to 0.5 per cent of vitamin C was injurious.

It is of interest to note that *T. interdigitale* and *T. discoides* both show deficiencies for thiamine and inositol. We found, however, no benefit from the addition of lactoflavin or pantothenic acid, both of which were beneficial to *T. interdigitale*. Mosher et al. did not determine the effect on *T. interdigitale* of pyridoxine, lack of which prevented growth of *T. discoides*. *T. interdigitale* differs from *T. discoides* also in ability to grow in the presence of any one of four vitamins. The latter organism required the presence of three vitamins in the medium, one or two of the three were not adequate. Mosher et al. (9) believe that *T. interdigitale* has "latent synthetic capabilities" and that "during a long growth period mechanisms are developed for the production of any one of a number of specific cell constituents which, under other conditions, may be taken from the environment as food." This did not seem to apply to thiamine, pyridoxine, and inositol for *T. discoides* as no growth occurred unless all three of the vitamins were present even when incubation was prolonged for more than 30 days.

Mosher et al. found various amino acids, especially leucine, hydroxy-α-amino butyric acid, aspartic acid, arginine, lysine, and phenylalanine, important in the nutrition of *T. interdigitale*. We obtained no benefit from the addition of numerous amino acids, including the six mentioned. On the other hand, the quantity of amino acids we used per culture was considerably

less than that used by Mosher et al. and a further investigation of the relation of *T. discoides* to amino acids is desirable. It is entirely possible that the unidentified favorable factors in peptone, casein, gelatin, and other natural substances are specific amino acids.

T. discoides is the only filamentous fungus known to the authors to have a complete inositol-deficiency. Eastcott (1) in 1928 found *i*-inositol to be a constituent of bios and important for the growth of a strain of yeast. Jansenns (4) reported inositol indispensable for the development of beer yeast. Williams and associates (15) found some strains of yeast to show a partial inositol-deficiency and others none. Some filamentous fungi have partial inositol-deficiencies (3, 6). Lash Miller, Kögl and Hasselt, and Wooley (7, 5, 18), found a high degree of specificity of inositol for yeast. Wooley (16, 17) reported inositol to be a growth factor for mice. Its deficiency resulted in stoppage of growth, paralysis of the hind quarters, and loss of hair. It seems clear that inositol is an important growth substance with a high degree of specificity, and further investigation will doubtless show deficiencies of various degrees in other organsms.

The complete inositol-deficiency shown by *T. discoides* suggests that this organism might be used for detecting and perhaps estimating inositol. However, before complete dependence can be placed on its use for the bio-assay of *i*-inositol further studies on specificity similar to those made on yeast should be made with this organism. The relatively slow growth of *T. discoides* and the need for an incubation temperature of 35° to 38° C. are disadvantages. Our own observations with this organism suggest that it may be useful in detecting the presence of inositol. For example, by bio-assay with *T. trichophyton,* it appears that agar contains little or no inositol while gelatin, casein hydrolysate, and albumen hydrolysate contain appreciable amounts.

SUMMARY

T. discoides suffers from complete deficiencies for pyridoxine, *i*-inositol, and molecular thiamine. In addition there are partial deficiencies for unidentified substances. The unidentified substances were present in peptone, casein hydrolysate, hydrolyzed egg albumen, malt extract, gelatin, and a filtrate (D_R fraction) prepared from white potatoes. No evidence was obtained that the partial deficiencies could be satisfied by biotin, lactoflavin, folic acid, pimelic acid, para-aminobenzoic acid, pantothenic acid, hypoxanthine, guanine, nicotinamide, 2-methyl-1,4-naphtho-quinone diacetate, seven pyrimidine and purine bases, and 43 amino acids. Ca phytate and a phosphatide containing inositol were less effective than *i*-inositol. The presence of appreciable quantities of inositol in gelatin, casein hydrolysate, and albumen hydrolysate was indicated by the growth of *T. discoides*. Unhydrolyzed agar appeared to contain little or no inositol. Maximum growth was obtained in a

4 weeks period in a medium containing between 0.1 and 0.5 mg. of inositol, and between 1 and 10 mμ moles of pyridoxine and thiamine.

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VARIATIONS IN ASCORBIC ACID AND DRY MATTER CONTENT OF COWPEA PLANTS AT DIFFERENT TIMES OF DAY

MARY ELIZABETH REID

The available data on variations in the ascorbic acid and dry matter content of cowpea plants at different times of day are presented here because of their possible bearing on the determination of a favorable time for harvesting fruits and vegetables and the special importance at this particular time of storing foods with the highest possible vitamin values. It has been recently pointed out by Auchter[1] that if we are to end with high nutritive values in the processing of fruits and vegetables, it is important that we start with them.

The observations herein reported were made during the growing seasons of 1939 and 1940 and were briefly mentioned in a previous publication (Reid 1942). It has been shown previously that losses of ascorbic acid occur at night (Reid 1939; Moldtmann 1939; Smith and Gillies 1940; Kohman and Porter 1940; Reid 1940, 1941). Moldtmann found fluctuations in the ascorbic acid content of leaves of deadnettles (*Lamium album*) with minimum values occurring at night and maximum values reached by mid-day or by 3 p.m. There was no significant difference in the vitamin C content of stems and petioles at different times. In fact, in these organs a small increase at night was found, but not always at the same time as the decrease in the leaves. With increasing light intensity and concomitant increase in assimilation synthesis of ascorbic acid was found to be favored, and to be depressed toward evening with the decreasing light intensity. No periodic variation was found in the ascorbic acid content of unripe fruits of elder (*Sambucus nigra*).

The literature dealing with the effect of seasonal and daily variations in light intensity upon ascorbic acid accumulation in plants has recently been reviewed together with the presentation of additional data obtained in a study with cowpea plants (Reid 1942). The interrelations of nitrogen and carbohydrates to ascorbic acid accumulation were discussed and it was suggested that weather conditions involving differences in light intensity should be considered in determining the time for harvesting fruits and vegetables. That a "time-of-day" factor should also be considered with respect to vitamin C values, particularly of leafy types of vegetables is strongly suggested by the present data.

[1] In a lecture given April 1, 1942, on "Interrelations of soil, plant, and human nutrition."

PROCEDURE

The very young plants employed in some of the tests were grown in sand without added nutrients; the older plants used in other experiments were cultured in glass jars containing nutrient solutions. A large number of cultures were prepared in most of the tests so that it was possible to select plants uniform in size and appearance for the ascorbic acid and dry weight determinations. At the time of transfer from the dark chamber and before synthesis of carbohydrates had begun, ascorbic acid assays were made of some of the plants and dry weight determinations were made of others. Similar determinations were made with other plants at different times during the day with a final determination toward the end of the light period. Five plants were used in each assay with very young plants and the same number for dry weight determinations. Two plants were used in each determination with the larger plants. Duplicate or triplicate evaluations were made in most of the tests. The values per plant as presented represent averages of the different groups of plants used in each test. Microchemical tests were made on thin sections of stem and leaf tissues for starch (iodine reaction) and reducing substances (Flückiger reaction).

OBSERVATIONS

The seven-day-old plants tested on May 24th were exposed to light of high intensity throughout each daily light period from the time of their emergence above the sand. A similar group of plants, ten days old, was tested on June 5, when the light intensity was also high throughout the day though it had been low during the four preceding days. The results of these experiments are shown in table 1.

The plants tested on May 24th showed significant gains in total dry weight and in total ascorbic acid until some time between 11 a.m. and 2 p.m., maximum values having possibly been reached before 2 p.m. No significant increase in ascorbic acid per unit of fresh weight occurred after 11 a.m. These plants contained a large amount of starch and reducing substances, a consequence of the shortage of mineral nutrients and high daytime illumination throughout the development of the plants. The plants tested on June 5th, on the other hand, gained throughout the day both in dry weight and in absolute amount of ascorbic acid as well as in ascorbic acid content on a green weight basis. Because of the preceding cloudy weather the plants started the day with a much lower content of dry matter and ascorbic acid. It is evident that on June 5th, when the light conditions became favorable for photosynthesis, the plants had the capacity to synthesize and accumulate both dry matter and ascorbic acid throughout the course of the day. In both tests the increase in ascorbic acid was much greater on a green than on a dry weight basis. In fact, on May 24th, a loss on a dry

TABLE 1

Ascorbic acid and dry matter content of two groups of plants at different times of day and exposed to different light intensities preceding the day of the tests

Time of day	Dry wt. per plt. (mg.)	Per cent dry matter	C per plt. (mg.)	C in fresh wt. (mg./g.)	C in dry wt. (mg./g.)
May 24 Very bright, preceding days also bright (Plants 7 days old)					
8:00 a.m.	136	8.7	0.727	0.441	5.35
11:00 a.m.	148	9.2	0.793	0.498	5.35
2:00 p.m.	159	9.5	0.822	0.502	5.17
5:00 p.m.	162	9.5	0.824	0.497	5.09
8:00 p.m.	160	9.3	0.822	0.501	5.14
June 5 Very bright, four preceding days cloudy (Plants 10 days old)					
8:00 a.m.	167	7.5	0.664	0.290	3.98
11:00 a.m.	180	7.9	0.686	0.302	3.81
2:00 p.m.	193	8.4	0.755	0.335	3.91
5:00 p.m.	195	8.6	0.824	0.345	4.23
8:00 p.m.	206	8.7	0.871	0.368	4.23

weight basis is indicated during the course of the day, a consequence of a more rapid increase in dry weight than in ascorbic acid.

The results of one of the tests with older plants are shown in table 2. The plants were grown in a partially shaded greenhouse and were placed in full sunlight in the open during the day preceding the test as well as on the day of the test. On the day of the transfer (June 12) the illumination was high until 1 p.m., after which it was cloudy for the remainder of the day. On June 13th, the day of the test, the light intensity was high until toward sunset. Gains in both dry matter and ascorbic acid occurred throughout the day. Because of having been grown in partial shade and with an abundance of mineral nutrients the plants had not previously accumulated much reserve carbohydrates and their ascorbic acid content was relatively low. In previous studies it had been found that the leaves of cowpea plants of this age grown under high illumination tend to have approximately 1 milligram of ascorbic acid per gram of fresh tissue; whereas, the leaves of the present plants contained only 0.66 milligrams of vitamin C per gram at the beginning of the light period and 0.80 milligrams at the end of the day. The stems gained in dry weight throughout the day but no appreciable increase in ascorbic acid was indicated after 11 a.m. The results suggest that the roots increased in total ascorbic acid and in total dry weight until 2 p.m. As in the previous tests the changes in ascorbic acid during the course of the day were much less on a dry than on a green weight basis. The 11

TABLE 2

Variations in green and dry weights and in ascorbic acid content of cowpea plants at different times of day

(June 13) Time of day	Green wt. per plant (g.)	Dry wt. per plant (g.)	Percentage of dry matter	Ascorbic acid		
				Total per plt. (mg.)	Mg./g. (green wt.)	Mg./g. (dry wt.)
Leaves						
8:00 a.m.	9.08	0.98	10.8	5.99	0.660	6.11
11:00 a.m.	9.22	1.06	11.5	6.66	0.722	6.28
2:00 p.m.	9.30	1.14	12.2	7.09	0.762	6.22
5:00 p.m.	9.60	1.27	13.2	7.72	0.804	6.08
Stems						
8:00 a.m.	7.44	0.63	8.5	0.84	0.113	1.33
11:00 a.m.	7.22	0.67	9.3	1.19	0.165	1.78
2:00 p.m.	7.11	0.66	9.6	1.22	0.170	1.85
5:00 p.m.	7.54	0.74	9.8	1.12	0.148	1.51
Roots						
8:00 a.m.	6.31	0.30	4.8	1.45	0.230	4.83
11:00 a.m.	6.44	0.31	4.8	1.65	0.256	5.32
2:00 p.m.	6.97	0.36	5.2	1.78	0.255	4.94
5:00 p.m.	7.21	0.36	5.0	1.70	0.236	4.72
Entire plant						
8:00 a.m.	22.8	1.92	8.4	8.27	0.362	4.31
11:00 a.m.	22.9	2.04	8.9	9.50	0.415	4.65
2:00 p.m.	23.4	2.16	9.2	10.09	0.431	4.67
5:00 p.m.	24.4	2.37	9.7	10.54	0.432	4.45

a.m. ascorbic acid values are higher than those at 8 a.m., but there were no consistent gains on a dry weight basis after this time.

Another set of slightly younger plants was tested on July 10th. This set also had been grown in a partially shaded greenhouse and was placed in the open in full sunlight two days previous to assaying. On July 10th the illumination was high until 3 p.m., after which it became cloudy (average radiation about 1800 ft.-c.). Because of having been exposed to full sunlight for a longer period than the plants tested on June 13th, these plants contained more starch and sugars and also had the same absolute amount of ascorbic acid but an approximately 20 per cent higher content on a green weight basis at the beginning of the light period, although the plants weighed approximately 20 per cent less than the June 13th plants. The total ascorbic acid increased from 8.17 milligrams at 5 a.m. to 10.20 milligrams at 3 p.m., a daylight increase of approximately 25 per cent. There was no increase in total dry weight and an apparent loss in ascorbic acid after 3 p.m. when it became cloudy (see table 3).

TABLE 3

Variations in green and dry weights and in ascorbic acid content of plants at different times of day

(July 10) Time of day	Green wt. (g.)	Dry wt. (g.)	Percentage of dry matter	Ascorbic acid		
				Total per plt. (mg.)	Mg./g. (green wt.)	Mg./g. (dry wt.)
5:00 a.m.	18.83	1.68	8.9	8.17	0.434	4.86
9:00 a.m.	19.21	1.77	9.2	8.63	0.449	4.88
12:00 m.	18.95	1.93	10.2	9.51	0.502	4.92
3:00 p.m. (Cloudy after 3:00)	18.80	1.97	10.5	10.20	0.542	5.18
6:00 p.m.	19.89	1.97	9.9	9.60	0.482	4.87

DISCUSSION

A definite influence of a light intensity factor on a "day-to-day" and even on a "time-of-day" effect upon the ascorbic content of cowpea plants is indicated by these results. In all of the tests conducted thus far on this problem, increases both in total amount of ascorbic acid per plant and in the ascorbic acid content per unit of fresh weight occurred upon exposure of the plants to light. In some tests, increases tended to occur throughout the day, whereas in others they ceased relatively early. In previous tests with plants used in other experiments it was found that the ascorbic acid values of plants grown in partial shade were relatively low even at the end of the day.

It is well accepted that carbohydrate synthesis is influenced directly by light intensity and indirectly by the supply of stored carbohydrates and availability of mineral nutrients, particularly of nitrogen. The concomitant increases in ascorbic acid and dry matter during the course of the day and from day to day suggest a close relation between the synthesis of carbohydrates and that of ascorbic acid. There is need for further work to determine their quantitative relations by more exact methods and the interacting influence of nitrogen supply.

SUMMARY

Progressive increases in both ascorbic acid and dry matter in cowpea plants are shown to occur from the beginning of the daily light period, and to continue during the course of the day depending upon the carbohydrate-synthesizing capacity of the tissues.

Plants grown under high illumination and much-limited nutrient supply tend to have relatively large reserves of starch and sugars and a low carbohydrate-synthesizing capacity. Under favorable light conditions such plants tend to attain their maximum vitamin C values early in the day.

Plants subjected to prolonged periods of cloudy weather, and especially if an abundant supply of mineral nutrients has been available, tend to have low vitamin C values and a low supply of starch and sugars.

The results suggest that for best vitamin C values, harvesting of vegetables should be done not before mid-forenoon after generally clear weather; or if it must be done following cloudy days, collection should be made as late in the day as possible.

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BULLETIN OF THE TORREY BOTANICAL CLUB
69(7): 528–538 OCTOBER, 1942

PHYCOLOGICAL NOTES—I

GEORGE J. HOLLENBERG

HAPALOSPONGIDION was established by Saunders (1899) on material collected at Pacific Grove, California. It is a brown alga which forms cushion-like small gelatinous thalli on rocks in the upper littoral zone. As described by Saunders, it is composed of a basal distromatic layer bearing erect closely packed and mostly unbranched filaments, which are free except at the tips, and which adhere on account of copious quantities of mucilaginous material. Saunders described both unilocular and plurilocular sporangia. The former he described as terminal and usually single, although he also described and figured series of enlarged cells in the erect filaments, which he took for immature or abortive unilocular sporangia. Largely on the strength of the latter observation, it seems, Setchell and Gardner (1924, 1925) doubted Saunders' description of single terminal unilocular sporangia and concluded that *Hapalospongidion gelatinosum* Saunders should be united with *Microspongium* under the name *M. saundersii* S. & G. In this disposition of Saunders' plant these investigators seem to have disregarded the fact that in the type species, *Microspongium gelatinosum*, described by Reinke (1888), both types of reproductive structures are laterally inserted on the erect filaments.

Saunders' plant (fig. 14) has been found by the writer at a number of places along the coast of southern California and near Carmel in central California. It has also been found by the writer as far south as Punta Banda, Lower California, Mexico. Examination of no. 583 of the Hancock Expedition of 1934, collected at Petillan Bay, Mexico, by Dr. W. R. Taylor, shows that it is the same as Saunders' plant.

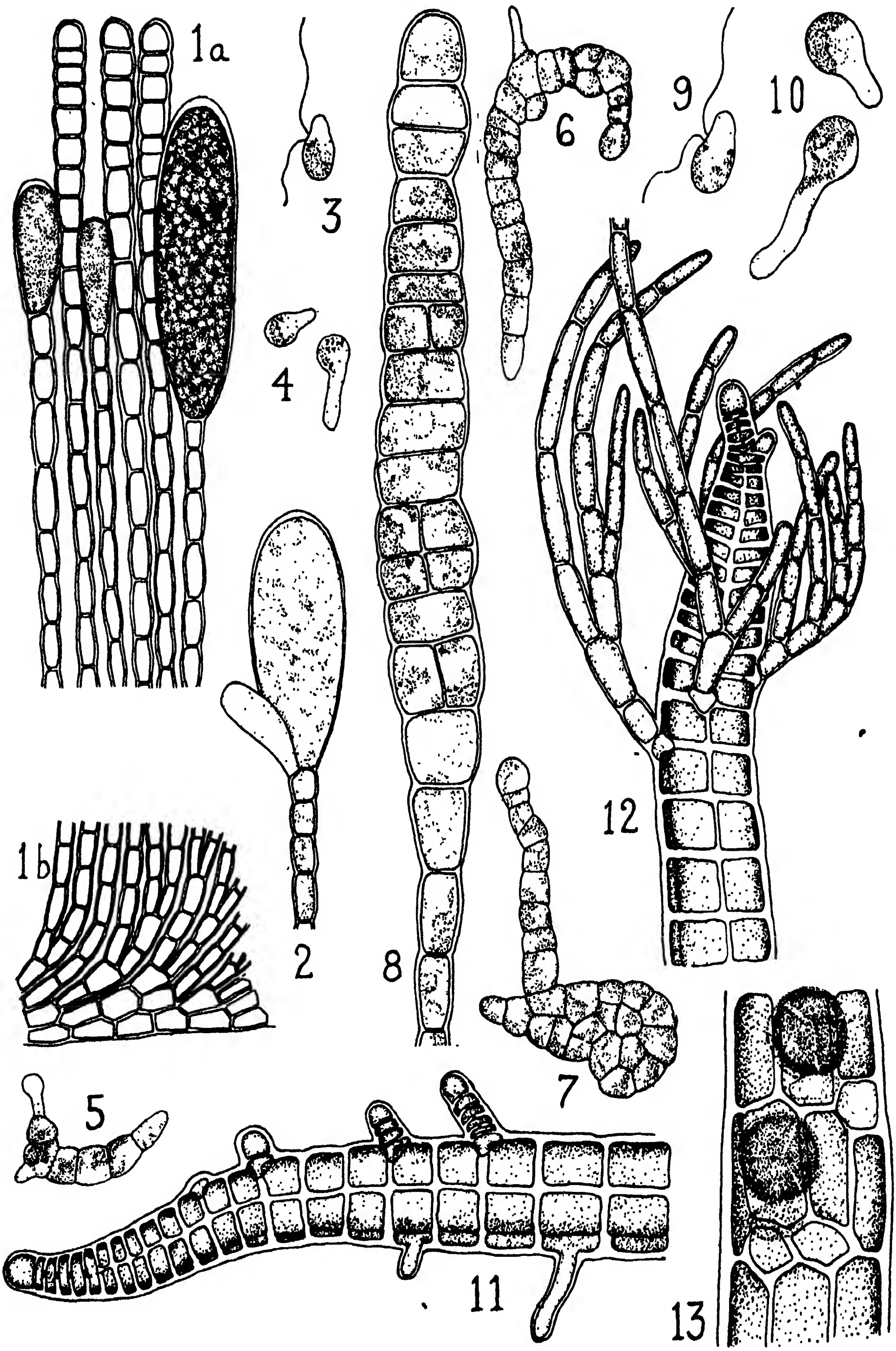
Near Corona del Mar in Orange County, California, the plant is usually abundant throughout the winter over an area several feet in diameter on a certain large rocky point. It has been observed at this locality for several successive seasons, and although it usually partially disappears during the winter months, it can be found at all seasons of the year. Both types of reproductive structures have been found a number of times. They correspond closely to Saunders' description and figures. Mature unilocular sporangia (fig. 1a) are relatively large, measuring 25–35 × 80–140 μ. They are oblong in shape and strictly terminal. They occur singly at the tips of the erect filaments. The tips of the sporangia are well below the tips of adjacent sterile filaments. No seriate intercalary unilocular sporangia were observed, and it seems probable that structures of such a nature reported by Saunders were abortive plurilocular sporangia. A few lateral outgrowths (fig. 2) were observed at the base of unilocular sporangia. These may be interpreted as

abortive accessory unilocular sporangia or possibly the true tip of the fruiting filament. In the latter case the unilocular sporangium could be thought of as actually lateral in position. A single very large nucleus is present in very young unilocular sporangia, suggesting that meiosis occurs in these reproductive structures as might well be expected, but no cytological studies were undertaken to determine the nature of the first nuclear divisions.

Normal plurilocular sporangia (fig. 8) occur on plants distinct from those which bear the unilocular sporangia, although both types of plants are often indistinguishably associated as a result of coalescence. The plurilocular sporangia are intercalary near the tips of the erect filaments, as described by Saunders. Their development is initiated by transverse divisions of cells immediately below the apical cell of a filament. One and usually two longitudinal divisions follow, resulting in the production of two tiers of four cells from each cell of the erect filament. These observations seem to necessitate retention of Saunders' genus *Hapalospongidion*, with *H. gelatinosum* as the type species.

Saunders does not describe motile reproductive cells of *Hapalospongidion* and did not study the development of germlings. The writer finds that zoids from both types of reproductive structures are of the usual form (figs. 3, 9), containing a single chromatophore and distinct stigma as well as two unequal flagella laterally inserted. Neither type of zoid gave evidence of fusing in the cultures. They were allowed to settle on cover-glasses and germling stages were studied in a manner similar to that employed for *Hapterophycus* (Hollenberg 1941). Examination of the cultures 12 hours after liberation of the zoids showed the formation of germ tubes (fig. 4). A single stigma was present at this time in germlings from both types of reproductive structures. It seems probable, therefore, that both types of zoids ordinarily germinate without fusing under culture conditions. The development of germlings from plurilocular sporangia was followed in the cultures for a period of one month. The entire contents of the germling initial usually migrates into the germ tube and is cut off from the old wall of the germling initial by a cross wall (fig. 5). Subsequent growth and cell divisions result in the formation of a short filament, usually provided with a single rhizoid. Longitudinal and irregular divisions, commonly accompanied by development of a sharp bend in the filament at the point of active cell division, soon initiate the development of a discoid thallus (figs. 6, 7). Although the development of the germlings was not followed beyond the development of this initial discoid thallus, it seems evident that the life cycle involves a facultative alternation of similar but distinct generations, parthenogenetic development of gametes seemingly repeating the haploid generation.

The writer has for some time been impressed with the similarity of the descriptions of certain other members of the Ralfsiaceae to *Hapalospon-*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gidion. *Mesospora* was described as a new genus by Madame Weber-van Bosse (1910), with *M. schmidtii* from the Malay archipelago as the type species. This plant was also found by the same investigator at several stations during the Siboga Expedition (Weber-van Bosse 1913). Later *Mesospora van-bossae* was described by Boergesen (1924) from Easter Island in the south Pacific. Still more recently Feldmann (1937) described *Mesospora mediterranea* from the Mediterranean. The writer has not been privileged to examine specimens of any of these species, but all are strikingly similar to *Hapalospongidion gelatinosum* in a number of important respects. In all of these plants the erect filaments are free except at the tips, where they are described as being united by a surface layer of the very gelatinous material surrounding the filaments. In all of these plants the plurilocular sporangia are intercalary, and separated from the tips of the filaments by one or more sterile cells, each cell in the fertile region dividing into the ultimate fruiting cells in a manner similar to that described by Saunders for the California plant. In all three plants the erect filaments arise from a basal stratum mostly two cells thick. In *M. mediterranea* Feldmann describes cell rows developing to some extent downward from this distromatic layer, and the writer has observed the same condition in *Hapalospongidion gelatinosum* in one instance. Unilocular sporangia are described as arising laterally at the base of erect filaments in the case of *M. schmidtii*, and Feldmann describes and figures lateral unilocular sporangia in the Mediterranean plant. These reproductive structures are not known in *M. van-bossae*. In *M. schmidtii* a series of large intercalary cells are described similar to those which Saunders interpreted as immature or abortive unilocular sporangia, but which as noted above are more probably abortive plurilocular sporangia.

The only important distinction between *Mesospora* and *Hapalospongidion* is the terminal position of the unilocular sporangia in the latter plant. However, as noted above, occasional unilocular sporangia bear lateral proliferations at the base, suggesting that in *Hapalospongidion* these reproductive

Explanation of figures 1–13

FIGS. 1–10. *Hapalospongidion gelatinosum* Saunders. FIG. 1a. Unilocular sporangia and paraphyses. × 500. FIG. 1b. Erect filaments of the thallus arising from the distromatic basal layer. × 500. FIG. 2. Immature unilocular sporangium showing rudimentary tip of filament (?). × 500. FIG. 3. Zoid from unilocular sporangium. × 1000. FIG. 4. Germinating zoids from unilocular sporangium. × 500. FIGS. 5–6. Germlings developing from zoids from plurilocular sporangia, one month old. × 500. FIG. 7. Germling from zoid from plurilocular sporangium, two months old. × 500. FIG. 8. Plurilocular sporangium at upper end of erect filament of thallus. × 1000. FIG. 9. Zoid from plurilocular sporangium. × 1000. FIG. 10. Germinating zoids from plurilocular sporangia. × 1000. FIGS. 11–13. *Lophosiphonia villum* (J. Ag.) S. & G. FIG. 11. Tip of prostrate branch showing rhizoids and adventitiously endogenous erect branches arising from the central cells. The latter are not shown. × 250. FIG. 12. Tip of erect branch showing arrangement of trichoblasts. × 250. FIG. 13. Portion of erect branch with tetrasporangia and double scar-cells. × 500.

structures may in some cases be thought of as essentially lateral. For the present it seems best, however, to retain *Mesospora* as a distinct genus.

A study of the type material of *Ralfsia pangoensis*, described by Setchell (1924) from Pagopago Harbor, Samoa, shows that this plant is a species of *Hapalospongidion*, with all the characteristic features of the genus, except that plurilocular sporangia are unknown. **Hapalospongidion pangoensis** (Setchell) Hollenberg, comb. nov., differs from *H. gelatinosum* chiefly in the shorter erect filaments, which are mostly under 200 μ long. The cells of the erect filaments are considerably shorter and more nearly cylindrical than in *H. gelatinosum*.

Pterochondria Hollenberg, gen. nov. Thallus polysiphonous, complanate, ecorticate, erect from a rhizoidal base embedded in the cryptostomata of *Cystoseira;* branching alternate, distichous, the branches slightly

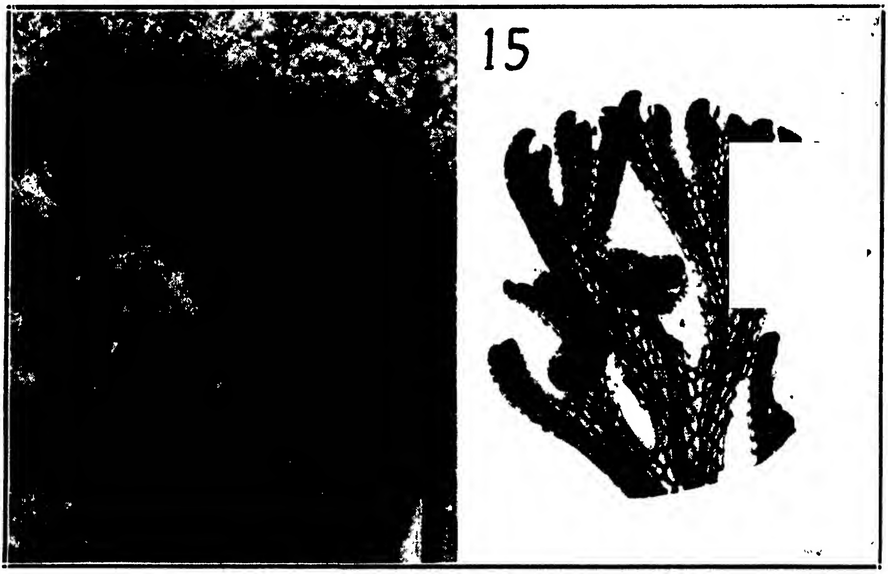

Fig. 14. Photograph of numerous plants of *Hapalospongidion gelatinosum* Saunders growing on a rock fragment. × 1. Fig. 15. Photomicrograph of a portion of *Pterochondria pygmaea* (Setch.) Hollenberg. × 30.

decurrent and of several to many orders, with mostly 3–5 segments between successive branches and with ultimate branchlets determinate; trichoblasts wanting; tetrasporangia one per segment, in straight series embedded in the adaxial side of the ultimate branchlets, tripartitely divided; antheridia covering the flat faces of flattened ovoid discs except for a sterile row of marginal cells; cystocarps globular ovoid, nearly sessile on the branches.

Thallus polysiphonus complanatus, ecorticatus, erectus ex fundamento radicato in cryptostomatibus *Cystoseirae* immerso; cum ramis alternantibus, distichis, aliquantum decurrentibus, ordinibus paucis aut multis, plerumque cum 3–5 segmentis inter ramos succedentes et cum ramulis ultimis determi-

natis; sine trichoblastis; tetrasporangiis singulis in segmentis in serie recta in latere adaxiale ramulorum ultimorum immersis, tripartite divisis; antheridiis superficies planas discorum complanatorum et ovatorum praeter ordinem sterilem cellulorum marginalium tegentibus; cystocarpiis ovoidoglobosis fere sessilibus in ramis.

There are two known species of *Pterochondria:* **P. woodii** (Harv.) Hollenberg, comb. nov., is the type species; *Pterosiphonia woodii* (Harv.) Falkenberg, Rhodomelaceen, 274. 1901; Setchell and Gardner, Algae Northwest. Am., 329. 1903; *Polysiphonia woodii* Harvey, Ner. Bor. Amer. 52. 1853. This species is commonly 15–20 cm. high and occurs commonly on *Cystoseira* from the vicinity of Monterey, California, to British Columbia. It seems to occur frequently on *Pleurophycus* stipes also.

Pterochondria pygmaea (Setchell) Hollenberg, comb. nov.; *Pterosiphonia pygmaea* (Setchell) Kylin, Calif. Rhodophyceen, 38. 1941; *Pterosiphonia woodii* f. *pygmaea* Setchell, Phycotheca Bor.-Am. no. *1744.* 1911. This is a diminutive species mostly under 2 cm. high, known only from southern California, where it occurs chiefly on *Cystoseira osmundacea* (Menz.) Ag., but is also reported as occurring on *Pelagophycus porra* (Leman) Setchell.

Two features distinguish this genus from all known species of *Pterosiphonia:* the discoid antheridial branches (fig. 15), and the lack of a cylindrical creeping base. A very similar male reproductive structure occurs in *Chondria* and also in *Acanthophora* (cf. Boergesen 1918). Falkenberg (1901) states that the antheridial branches of *Pterosiphonia dendroidea* (Mont.) Falk. are cylindrical structures similar to those of *Polysiphonia.* The writer has observed male reproductive structures in several species of *Pterosiphonia* along the California coast. In all these species the antheridial branches seem to be cylindrical. It seems probable that this is true of all true species of *Pterosiphonia.* Falkenberg (1901) figures discoid antheridial branches for *Polysiphonia virgata* but it seems improbable that this will prove to be an accurate observation concerning a species of *Polysiphonia.*

Concerning the attachment organs of *Pterochondria*, F. S. Collins, in an unpublished manuscript on the Rhodomelaceae of North America, states that the spores germinate in the cryptostomata of *Cystoseira osmundaceae,* and that the young filaments are at first monosiphonous, but become polysiphonous soon after emerging from the cryptostomata.

The bluntly uncinate tips of determinate branchlets, especially evident in the case of young ramuli, seem characteristic of *Pterochondria.* This condition is seemingly due to a much retarded development of pericentral cells on the inner side of the branchlets. This also seems to account for the beaklike appearance of apices of branches, and possibly also for the staggered arrangement of the pericentral cells of a branchlet with respect to those of

the axis in the region where the branchlet is decurrent on the axis. Harvey (1853) mentions the latter condition indirectly in his original description of *Polysiphonia woodii,* when he speaks of the pericentral cells as being arranged in two axes.

TAENIOMA CLEVELANDII Farlow. *Taenioma perpusillum,* the type species, was originally described by J. G. Agardh (1851–1863) from material collected on the Pacific Coast of Mexico by Liebmann. The plant has since been collected from a number of places including points along the Atlantic coast of North America and in Europe. There was a difference of opinion whether the plant should be placed in the Delesseriaceae or the Rhodomelaceae until Thompson (1910) described the morphology and sexual reproductive structures and showed that the plant is a member of the Rhodomelaceae. *Taenioma clevelandii* was described by Farlow (1877) from material collected by Cleveland at San Diego, California. As pointed out by Farlow, the California plant is much larger, up to 10 cm. high, and lacks the fasciculate branching of *T. perpusillum.* However, De Toni (1924) makes *T. clevelandii* a synonym of *T. perpusillum,* without giving reasons for so doing. In the herbarium of the University of California are several specimens of *Taenioma* collected along the coast of California from San Diego to Central California. During the summer of 1941 the writer collected this relatively rare plant afloat near Pacific Grove, California. Examination of this material, which was tetrasporic, and the other specimens in the herbarium of the University of California, one or more of which bore tetrasporangia, but none of which bore sexual reproductive structures, has led the writer to conclude that *T. clevelandii* is amply distinct from *T. perpusillum.* It differs not only in size and lack of fasciculate branching, but in several more distinctive respects. In the first place many segments usually intervene between successive branches in *T. clevelandii,* whereas relatively few segments occur between successive branches in *T. perpusillum.* Furthermore, the branches do not end in hairs in any of the specimens of *T. clevelandii* examined by the writer. Farlow failed to mention two additional points of difference. *T. clevelandii* has a rhizome as *T. perpusillum* has, but the flanking cells, which in the genus *Taenioma* are marginal in position on the flattened branches, and which are only half as long as the pericentral cells, occur on all branches, including the basal rhizome, in *T. clevelandii,* rather than on the ultimate branches only as in *T. perpusillum.* Finally, rhizoids are all unicellular in the specimens of *T. perpusillum* available for examination, whereas those of *T. clevelandii* are usually composed of two cells. In *T. clevelandii* branches arise from the center of the flat faces of the axes, presumably from the central cell.

GELIDIUM CALOGLOSSOIDES Howe. This species was originally described by Howe (1914) from Peru, and seems not to have been reported in the

literature since. During the past several years the writer has repeatedly encountered a diminutive creeping species of *Gelidium* along the coast of southern California and several times also in the Monterey region in the central part of the state. Examination of type material shows that these plants must be placed with Howe's species, from which they differ only in the lack of dwarf ventral branchlets near the hapterous attachment organs, which Howe described as frequently present in the Peruvian plant, and which the writer observed in the type material. Examination of additional material from Peru will be necessary to determine whether or not this is a constant difference. Tetrasporangia occur in diagonal rows in the lateral and finally somewhat erect branchlets as in the Peruvian plant. The internal structure is also very similar.

LOPHOSIPHONIA VILLUM (J. Ag.) S. & G., figs. 11–13. *Lophosiphonia villum* (J. Ag.) Setchell and Gardner, Algae Northwest. Am. *329*. 1903; Kylin, Lunds Univ. Årsskr. N. F. Avd. 2. **37**: 40, 1941. *Polysiphonia villum* J. Agardh, Sp. Alg. II. 941, 1863. Plants erect, 5–10–(18) mm. high, from prostrate creeping branches 40–60–(80) μ diam., of segments 1–1.5 diam. long, without trichoblasts, attached by frequent unicellular rhizoids with expanded lobed tips; rhizoids arising as outgrowths from the middle of the pericentral cells from which they are not cut off by a cross wall; erect branches simple or sparingly branched 40–80–(100) μ diam., of segments 1–2 diam. long, arising at irregular intervals mostly every 2–4 segments in a strictly endogenous manner, at first arching toward the tips of the prostrate branches; lateral branches arising from erect branches exogenously or endogenously, independent of the trichoblasts which they may replace; pericentral cells 4, totally without cortication; chromatophores forming transverse bands in the pericentral cells; trichoblasts mostly infrequent and often wanting, but sometimes relatively abundant, irregular in occurrence but tending to occur one per segment in a left hand spiral with one fourth divergence when abundant, once or twice or sometimes thrice forked, rather stiff, often recurved or even coiled, 15–20 μ diam., 250–480 μ long, soon deciduous, leaving persistent scar-cells which sometimes divide to form double scar-cells (fig. 13); tetrasporangia 50–60 μ diam. one per short swollen segment in more or less continuous straight series; antheridial branches cylindrical, incurved, arising from the entire branch primordium, 70–170 × 20–35 μ, on short one-celled pedicels, without sterile tips; cystocarps ovoid, sessile, more or less erect, 150–190 μ diam.; plants dark reddish brown, attached in small tufts or forming a continuous stratum on rocks and other algae, often intermingled with other mat forming algae, frequent mostly in the middle and upper littoral zone, central California to tropical America.

This plant has been collected by the writer about 20 different times, mostly along the coast of southern California. In general the plants agree well with the original description by J. Agardh (1863). Professor W. A. Setchell has kindly allowed the writer to examine some unpublished notes

which he made while examining types in European herbaria. Among these is a note to the effect that no trichoblasts are present in the type material of *Polysiphonia villum* in the Agardhian herbarium. However, the writer finds that the presence of trichoblasts is a variable condition in this plant, as is also the degree of branching of erect branches. The plant is readily recognized locally by its diminutive size, and by the relatively little branched or unbranched erect branches arising endogenously from prominent and permanently prostrate branches. These characters seem to warrant placing the plant in *Lophosiphonia* as Setchell and Gardner (1903) and Kylin (1941) have done. However, in this plant, as in certain other species of *Lophosiphonia* and *Polysiphonia*, the vagueness of the characters distinguishing these two genera is most vexingly evident.

According to Falkenberg (1901) the most characteristic features of *Lophosiphonia* are: (1) relatively limited and mostly simple or sparingly branched erect branches from distinctly and permanently prostrate branches; and (2) adventitiously endogenous origin of all branches. Examination of several specimens of *Polysiphonia hemisphaerica* Aresch. from the Baltic Sea indicates that in this plant all branches arise endogenously and adventitiously, but the erect branches are several inches high and repeatedly branched. Further study may necessitate removal of this plant from the genus *Polysiphonia,* but it certainly cannot be placed in the genus *Lophosiphonia.* In *Lophosiphonia villum* all erect branches and most of the lateral branches to which they give rise are produced in an adventitiously endogenous manner. However, careful examination shows that lateral branches frequently arise exogenously by division of subapical cells. A further complicating feature is the fact that in *Lophosiphonia villum* the rhizoids arise from the prostrate branches as outgrowths of the pericentral cells from which they are not cut off by a cross wall. In this respect as well as the origin of antheridial branches from the entire trichoblast primordia, *L. villum* is closely similar to a species of *Polysiphonia* common along the Pacific Coast of North America, which has been commonly identified as *P. urceolata* (Lightf.) Grev., but is seemingly a distinct species. Furthermore, it should be noted in this connection that in a number of local species of *Polysiphonia* erect branches frequently arise endogenously from distinctly prostrate branches.

The foregoing considerations make it seem necessary to conclude that, if the genus *Lophosiphonia* is to be retained at all, its characteristic features need to be more clearly delimited if possible. Accordingly it is proposed that the concept of the genus expressed by Falkenberg (1901) be modified slightly so as to include only those Rhodomelaceous plants with a single tetrasporangium per segment, which have relatively prominent and strictly prostrate branches giving rise to erect branches in a strictly adventitious and

endogenous manner, and in which the erect branches are relatively short and simple or bear relatively few lateral branches in an adventitious and *mostly* in an endogenous manner. This characterization differs from Falkenberg's concept only in that it provides that some of the lateral branches may arise exogenously from the erect branches. It is admittedly relative, perhaps even more so than Falkenberg's concept of the genus, but this modification seems necessary if the genus is to be retained at all. There will probably continue to be some confusion concerning the limitations of the genus, but if one should adhere strictly to Falkenberg's concept of the genus, *Lophosiphonia villum* would need to be returned to the genus *Polysiphonia,* in spite of its habit, since not all branches arise endogenously. This would perhaps be more confusing than helpful. Hence it has seemed best to conclude with Setchell and Gardner that it belongs in *Lophosiphonia.*

SUMMARY

1. *Hapalospongidion* should be recognized as a valid genus in the Ralfsiaceae.

2. *Pterochondria* is described as a new genus differing from *Pterosiphonia* in the flattened discoid antheridial branches similar to those of *Chondria* and in the lack of a creeping base.

3. *Taenioma clevelandii* Farlow is a valid species distinguished from *T. perpusillum* J. Ag. chiefly by the occurrence of the characteristic short flanking cells on all branches, including the prostrate rhizome, and by the rhizoids which are composed mostly of two cells rather than of one as in the type species.

4. *Gelidium caloglossoides* Howe occurs frequently along the coast of southern California.

5. *Lophosiphonia villum* (J. Ag.) S. & G. is somewhat intermediate between *Lophosiphonia* and *Polysiphonia.* Its inclusion in the first named genus seems to require a slight modification of Falkenberg's concept of the genus.

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PANICUM BENNETTENSE, A NEW SPECIES FROM NORTH CAROLINA

WALTER V. BROWN

While botanizing near Durham, North Carolina, a panicum was collected which at first was taken for *P. angustifolium.* On closer examination, however, it was seen that it differed from that species in a number of particulars although belonging to the section Angustifolia. Professor H. L. Blomquist of the Department of Botany, Duke University, after a careful study of the material feels that it is an undescribed species. J. R. Swallen of the U.S.D.A. also expressed the view that it is a new species.

Panicum bennettense, W. V. Brown, sp. nov. (subgenus *Dicanthelium* Hitchc. & Chase, group *Angustifolia*). Planta caespitosa, 30–70 cm. alta; internodiis productis, 5–7, puberulis vel papillati-pubescentibus; nodis glabratis; vaginis sparse adscendenti-papillati-pubescentibus, superioribus tamen glabratis; statu vernali foliis 8–15 cm. longis, 4–8 mm. latis, planis, in longitudine sulcatis, productis acuminatisque, rigidis, ad basim paulo angustatis et ciliatis; foliis inferiorbus rigiduli-pubescentibus, superioribus scabris ad apicem involutam; ligulis ciliatis, 1–1.5 mm. longis; paniculis vernis longe exsertis, 5–9 cm. longis, 4–9 cm. diam.; rhachidibus glabratis; ramis late extensis, deinde postea adscendentibus; pedicellis productis, glabris; spiculis pubescentibus, 2 mm. longis, 1.2 mm. diam. ellipticis, apice acuminato, gluma inferiore 0.5 mm. longa, superiore et lemmate sterili aequilongis; fructu 1.7 mm. longo, 1.0 mm. diam.; statu autumnali ad basim rare ramoso sed ad nodos mediales atque superiores sparse ramoso, ramis adscendentibus, paniculis terminalibus, 1–2 cm. longis.

NORTH CAROLINA: Durham County, dry thickets in savannah at the Bennett Memorial, 5 miles west of Durham. TYPE in the Duke University Herbarium, *W. V. Brown 2492.*

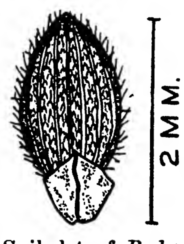

FIG. 1. Spikelet of *P. bennettense*

Plant 35–70 cm. high, erect, the lowermost internodes crisp papillose-pubescent, the middle and upper sparsely puberulent, the nodes glabrous; lower sheaths ascending-papillose-pubescent, the upper glabrous, ciliate; blades 8–15 cm. long, 4–8 mm. wide, stiff, flat, long acuminate, scarcely narrowed at the usually ciliate base, the lower often longitudinally wrinkled and shorter, harsh pubescent, the upper scabrous especially at the involute

tip; ligule 1–1.5 mm. long; panicle long exserted, 5–9 cm. long, 4–9 cm. in diameter, wide spreading at anthesis, later ascending, the axis glabrous; spikelets 2.0 mm. long, 1.2 mm. in diameter, ellipsoid, slightly attenuate at base, acuminate at tip, turgid; first glume about $\frac{1}{4}$ as long as the spikelet, glabrous, shining, pointed; second glume and sterile lemma equal, papillose-pubescent; fruit 1.7 mm. long, 1.0 mm. in diameter, elliptic. Autumnal culms stiffly ascending, sparsely branched at the middle and upper nodes, the branches ascending; blades numerous, flat, slightly involute at the long acuminate tip, appressed, thin; secondary panicles terminal, 1–2 cm. long, often reduced to 3 or 4 spikelets.

This species is very close to *P. angustifolium* Ell., differing mainly in spikelet characters. The spikelets of *P. angustifolium* are 2.5–2.8 mm. long, 1.4–1.6 mm. in diameter, obovate, blunt, the first glume $\frac{1}{3}$ the length of the spikelet. *P. bennettense* has spikelets 2.0 mm. long, 1.2 mm. in diameter, elliptic, pointed, the first glume $\frac{1}{4}$ the length of the spikelet. The ligule is longer and the branches of the inflorescence more ascending than in *P. angustifolium.*

Three stools of this species were collected on the dry, sandy, savannah-like park surrounding the Bennett Civil War Memorial. This park has a grass flora rich in species of *Panicum*, 30 or more mostly coastal-plain forms having been collected in the four or five acres. It is for this place, 5 miles west of Durham, that the species has been named.

Herbarium specimens were made of the three plants and given numbers *2492A, 2517,* and *2472*. Half of the stool of number *2492A* was potted and grown to the autumnal phase. This was then made into herbarium specimens and numbered *2492B*. The description is based on these four collections. A portion of *2492A* in the vernal phase was chosen as the type, was so marked, and has been deposited in the Duke University Herbarium. Co-types are numbered *2492A* and *2492B*, consist of both vernal and autumnal phases, and are from the same plant as the type. Isotypes are numbered *2472* and *2517* and are specimens of plants in the vernal phase. Complete sets of these specimens have been deposited in the Duke University Herbarium, the U. S. National Herbarium, and the author's personal herbarium.

The writer wishes to thank Professor H. L. Blomquist and Dr. Jason R. Swallen for their opinions concerning the status of these plants; Professors F. A. Wolf and R. S. Rogers for help with the Latin diagnosis, Professor H. J. Oosting and Dr. Lewis E. Anderson for help in preparing the manuscript.

DEPARTMENT OF BOTANY, DUKE UNIVERSITY
DURHAM, NORTH CAROLINA

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THREE NEW CUSCUTAS FROM WESTERN NORTH AMERICA

T. G. YUNCKER

Cuscuta Runyonii Yuncker, sp. nov. Caules tenues aut modici. Flores 2–3 mm. longi a basi ad corollae sinum, glandulosi. Pedicellati aequantes aut longiores quam flores. Inflorescentiae umbellato-cymosae. Calycis lobi triangulares aut plus minus ovati, subacuti, appendiculati-corniculati. Corollae lobi ovato-lanceolati, acutissimi, tubo campanulato aut subgloboso aequilongi, reflexi, apices inflexi. Squamae fimbriatae ad stamina attingentes. Styli tenui, aequantes aut longiori quam ovarium depresso-globossum. Capsula depresso-globosa, non circumscissilis. Semina 4, ovala, circ. 1.5 mm. longa.

Stems slender to medium, smooth or often with scattered papillae near the inflorescence. Flowers "white" or "cream-white," drying yellow or reddish, 5-parted, mostly 2 to 3 mm. long from the base of the flower to the corolla sinuses, glandular, on pedicels varying in length, but commonly about equal to or somewhat longer than the flowers, in loose, umbellate cymes of 2 to 5 or 6 flowers, these ultimate clusters also sometimes umbellately arranged, bracts mostly 1 to 1.5 mm. long, oblong-ovate to triangular, acute, with a short, obtuse, saccate protuberance near the base. Calyx lobes triangular to somewhat ovate, obtuse or acutish, not overlapping at the base, about reaching the corolla sinuses, or shorter, each with a prominent, spur-like protuberance at the base and also frequently with a somewhat smaller one towards the apex. Corolla campanulate to subglobose, smooth or more or less scabrous, somewhat bulging between the lines of the filament attachments, lobes about as long as the tube, triangular-ovate to lanceolate, reflexed, acute to acuminate, tips inflexed, papillate. Stamens shorter than the corolla lobes, filaments slightly subulate and about equal to or slightly longer than the oval anthers. Infrastamineal scales prominent, reaching the stamens, profusely fimbriated, bridged below the middle. Styles slender, equal to or longer than the depressed-globose ovary, stigmas capitate. Capsules depressed-subglobose, smooth or papillate, not circumscissile, surrounded by the corolla. Seeds about 1.5 mm. long, oval, 4 in each of the capsules examined, embryo filiform, coiled.

TEXAS: Hidalgo County, occasional on dry clay hilltops, at La Joya, June 8, 1941, *Robert Runyon 2732*, TYPE, U. S. National Herbarium; occasional on dry clay hills, chaparral thickets, 2 miles north of La Joya, July 13, 1941, *Robert Runyon 2825*. (Very abundant material of the above two specimens permitted division and duplicates have been deposited in the Gray Herbarium, New York Botanical Garden, Field Museum of Natural History, and the Missouri Botanical Garden); Zapata County, 3¾ miles north of San Ygnacio, April 1, 1938, *V. L. Cory 28121* (Gray); Duval County, 21 miles northwest of San Diego, October 9, 1936, *V. L. Cory 17199* (Gray).

This species is remarkable because of the spurred calyx. The spurs, which have been observed on no other species, are sufficiently prominent to be readily visible with the unaided eye. They are mostly simple, sac-like protuberances tapering to an obtuse tip, but several were noted in which the

spurs were branched. They are nearly as wide at the base as the calyx lobe, or commonly narrower. Examination of hundreds of flowers failed to reveal any which were without spurs. Host plants include *Calophanes decumbens, Erigeron canadensis, Coldenia canescens,* and *Nama hispida.*

This species belongs in Subsection *Arvenses* of Section *Cleistogrammica* and appears to be most closely related to *C. glabrior* (Engelm.) Yuncker, a common species of the southwestern United States and northern Mexico. The prominently spurred and somewhat more triangular lobes of the calyx readily distinguish it from that species, however. From *C. appendiculata* Engelm. of South Africa it differs in its larger flowers, much larger projections on the calyx, character of the inflorescence, etc. It is named for Robert Runyon, collector of the type specimen.

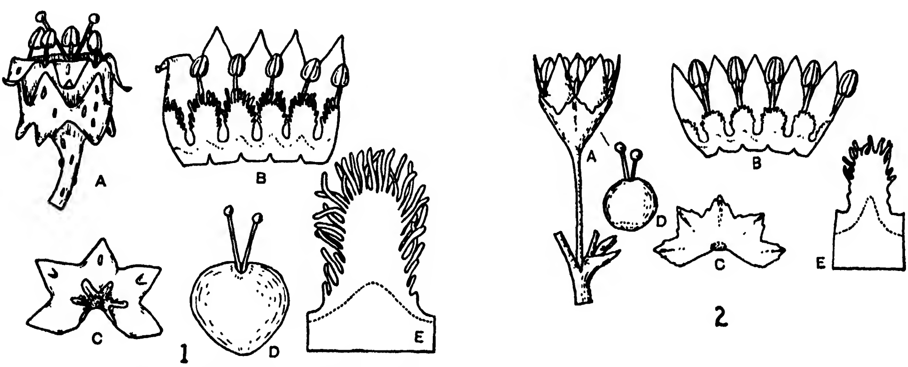

FIG. 1. *Cuscuta Runyonii* Yuncker, sp. nov. FIG. 2. *Cuscuta serruloba* Yuncker, sp. nov. *a,* flower × 10; *b,* opened corolla × 10; *c,* opened calyx × 10; *d,* fig. 1, capsule × 10; *d,* fig. 2, ovary × 10; *e,* individual scale × 20.

Cuscuta serruloba Yuncker, sp. nov. Caules tenuissimi. Flores 4- aut 5-divisi, circ. 1.5 mm. longi a basi ad corollae sinum. Pedicellati longiores quam flores. Calycis lobi triangulares, acuti, irregulares serrati. Corollae lobi oblongo-lanceolati, plus aut minus acuti. Scalae oblongae, fimbriatae, ad stamina attingentes. Filamenta plus aut minus subulati. Styli subulati breviores quam ovarium subglobosum. Capsula non visa, circumscissilis (?).

Stems very slender. Flowers 4- or 5-parted, about 1.5 mm. long from the base of the flower to the corolla sinuses, on pedicels mostly much longer than the flowers, in loose, mostly 3- to 5-flowered umbellate cymes, these in turn more or less paniculately arranged, bracts ovate, acute, denticulate. Calyx somewhat fleshy, lobes deltoid, not overlapping, subequal, medianally thickened and slightly crested near the acute apex, about reaching the corolla sinuses, edges irregularly toothed. Corolla lobes oblong-lanceolate, acutish, about as long as the campanulate tube, erect to spreading, margins entire or obscurely uneven. Stamens nearly as long as the corolla lobes, filaments stoutish, enlarging somewhat towards the base, commonly as long as or slightly longer than the comparatively large, oval-ovate anthers. Infrastamineal scales reaching the stamens, oblong, moderately fringed, bridged at about the middle. Styles stoutish and enlarging slightly toward the base, shorter than or about equal to the subglobose ovary, stigmas comparatively large, subglobose, smooth. No matured capsules are present on the material

examined but a line near the base of the ovary indicates that they are probably circumscissile.

MEXICO—COLIMA: Manzanillo, October 21, 1910, *C. R. Orcutt 4457*, TYPE, Gray Herbarium.

The material from which the above description is drawn is not abundant and does not contain any matured fruit. It appears evident, however, that this species belongs in Subsection *Umbellatae* of Section *Eugrammica*. It is apparently most closely related to *C. deltoidea* Yuncker, the type of which was collected in the same locality. It differs from that species, however, in the acute, non-overlapping and more triangular, serrulate calyx lobes, proportionately longer corolla lobes and the shorter (?) and stouter styles. From *C. lacerata* Yuncker it differs chiefly in the shape of calyx and corolla lobes and from *C. gracillima* Engelmann because of its shorter stamens and serrate calyx lobes.

CUSCUTA SALINA Engelmann var. **papillata** Yuncker, var. nov. Calycis et corollae lobi ovato-lanceolati, attenuati, acutissimi. Calycis et pedicellis superior pars papillosa.

Flowers about 3 mm. long from the base of the flower to the corolla sinuses. Calyx and corolla lobes ovate-lanceolate, attenuately acuminate. Calyx and upper part of the pedicel finely papillate. Anthers ovate-subsagittate, filaments subulate. Infrastamineal scales represented by narrow, sparingly fringed ridges up to about the middle of the corolla tube.

CALIFORNIA: Mendocino County, Fort Bragg, August 8–16, 1912, *Alice Eastwood 1593*, TYPE, Gray Herbarium.

This variety resembles the species in most respects. It differs principally in the papillate calyx and more attenuate perianth lobes.

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INDEX TO AMERICAN BOTANICAL LITERATURE

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Papers that relate exclusively to bacteriology, forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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PLANT TAXONOMY AND FLORISTICS
(exclusive of fungi)

(See also under Genetics: **Cave & Constance, Clausen, Epling & Dobzhansky, Góes**)

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THE RESPONSE OF ACHRAS ZAPOTA IN LATEX YIELD TO WOUNDING BY THE IBIDEM[1] METHOD OF TAPPING

JOHN S. KARLING

Among the three outstanding characteristics of *Hevea braziliensis* which have made this species the principal source of rubber is its marked positive response in the form of increased latex yield to the stimulus of tapping injury or wounding. When trees are first tapped the yield is comparatively slight, but as a response to daily wounding it increases steadily and eventually reaches a climax. This response as well as a rapid growth rate and the fact that the trees may be bled daily for long periods of time make *Hevea braziliensis* ideal for plantation cultivation and are largely responsible for the vast development of the present-day rubber industry.

In the light of these facts preliminary experiments were made at the start of our Chicle Research Project, under the auspices of the Tropical Plant Research Foundation and the Chicle Development Company, in British Honduras to determine whether or not *Achras zapota,* the principal source of chicle, can be tapped daily and will respond to wounding to the same degree as *Hevea braziliensis.* For these experiments six healthy sapodilla trees were selected near the central camp at Tower Hill, and panels were marked off on the boles for tapping. These trees had a thick, hard, ridged outer bark which was impregnable to the tapping gouges at hand at that time, and in order to tap the trees it was necessary to remove the outer bark completely with a machete. This removal naturally led to marked desiccation of the softer cortical layers during the hot middays, but this drying out was checked by nailing heavy burlap sacks above the panels which could then be let down as soon as tapping and drainage of the latex were completed. These sacks were wetted thoroughly every day, so that the outer layers of the exposed cortex were kept moist.

Tapping began by making an oblique cut or channel at an ascending angle of approximately 40° halfway around the bole, and during the successive daily tappings the lower margin of the initial channel was pared off to reopen the ends of the latex tubes. The gouges used in these initial tests were crude, and it was impossible to remove less than one-eighth inch of bark with each successive paring. For this reason bark consumption in a vertical direction was considerably higher than in tapping *Hevea* (Maas 1926). Daily tapping continued over a period of three weeks and was then

[1] Ibidem, meaning "in the same place" is a descriptive name proposed by Cook (1928) for the present-day method of tapping *Hevea braziliensis,* in which the lower margin of the initial incision is continually pared off during successive tappings.

suspended because of the difficulty of making clean-cut incisions. The results of these preliminary tests in grams of bone-dry chicle were as follows:

Daily yield in grams of bone-dry chicle

Tree no.	Girth	1st day	2nd day	3rd day	2nd week	3rd week
1	3′ 2″	42	0.0	0	0	0
2	2′11″	38	1.80	0	0	0
3	2′10″	26	0.89	0	0	0
4	3′ 8″	52	0.0	0	0	0
5	3′ 1″	35	0.0	0	0	0
6	2′ 9″	40	0.0	0	0	0

The initial yield for the first day is quite high and compares favorably in proportion to tree girth with that of *Hevea braziliensis* as shown by the results obtained by Heusser (1924–1930), Polhamus (1928), Holder and Heusser (1928), Heusser and Holder (1929–1930), Schmole and Prummel (1930), and others. However, with the exception of trees 2 and 3 the yields were negative on the succeeding days. In most panels a few drops of latex accumulated on the pared surface of the oblique incisions if the incisions were accidentally made deeper and uncut latex vessels were severed, but the yield was never of sufficient quantity to be collected. These results suggested at once that *Achras zapota* does not respond to the stimulus of slight daily wounding and cannot be profitably tapped daily in the same manner as *Hevea braziliensis*. As a consequence our time and efforts were concentrated on wound stimulus experiments of another type and on modifying and improving the native method of tapping.

However, in the ensuing years since these initial experiments were performed we felt that the results obtained were not conclusive, inasmuch as the tapping gouges used did not make clean cut incisions. Since that time we have modified the gouge or knife commonly used in tapping *Hevea braziliensis* and made it more suitable to the harder bark of *Achras zapota*. For these reasons it has become desirable during the subsequent years to duplicate our initial ibidem experiments and determine more conclusively the wound stimulus possibilities of the sapodilla tree.

Accordingly, a similar experiment was performed on thirteen trees growing in a cohune palm grove back of the "milpa" at Tower Hill. Young trees with smooth straight boles and a thin outer bark were selected to avoid removal of the outer corky layers before tapping. These trees stand in a dense cohune palm grove bordering Doubloon Bank Savannah where the ground is always damp during the rainy season. Through this grove flows a small sluggish stream which overflows its banks and floods the entire region after heavy rains. As a consequence it is not uncommon to find standing water around the base of the trees for several days after a rainstorm. The dense growth of palms and other trees keep out most of the sunlight and wind, so that relative humidity and tree turgidity (Karling 1934) are quite

high throughout the day. External environmental conditions in this region are thus conducive to maximum yield and sufficiently constant to eliminate most of the effects of transpiration, loss of turgidity, latex coagulation in the incisions, etc., during the period required for tapping and draining of the latex. Tapping began before 5 o-clock a.m. and was usually completed shortly before sunrise. Quite often the work began with the aid of a flashlight, since most of the early dawn light was excluded by the dense growth of palms. With the exception of only two mornings, relative humidity was one hundred per cent during the tapping period and for several hours thereafter.

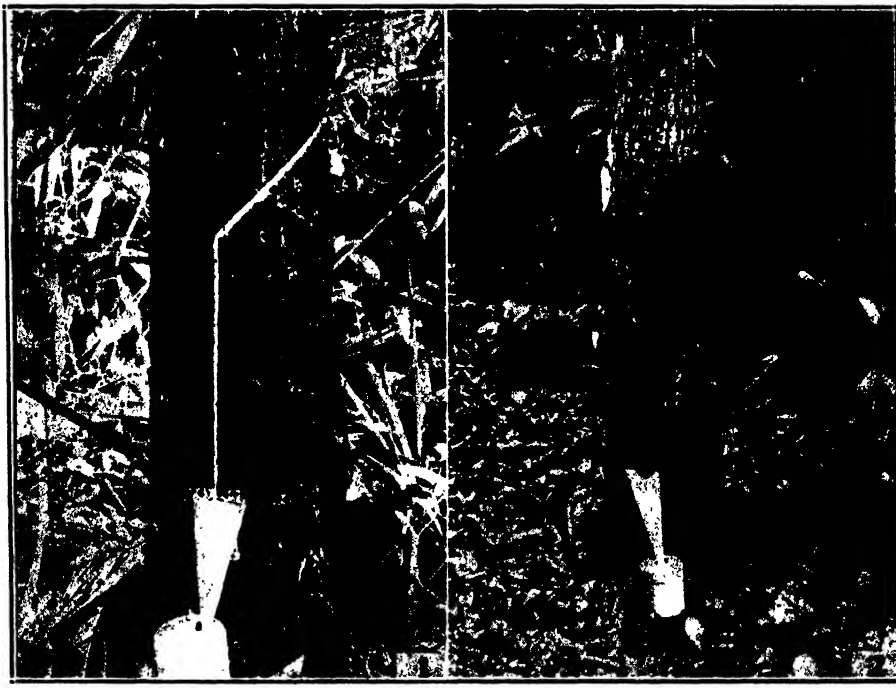

FIG. 1. Tree 9 showing the manner of opening up the ibidem panels on the first day of tapping. FIG. 2. Tree 1 after 14 vertical inches of bark had been removed.

Each panel was opened approximately three feet above the ground, and the oblique channels extended half-way around the bole as is shown in figures 1–4. The latex was collected separately from each tree in paper cartons, coagulated, dried, and finally analyzed for moisture content. The improved highly-tempered and sharp gouges cut through the outer bark quite easily, severed the latex tubes cleanly, and made perfect oblique channels for the flow of the latex. The depth of tapping extended to within a few millimeters of the cambium, as is shown in table 1, and was kept as uniform as possible throughout the experiment. As in the preliminary tests, the successive daily

tappings were made by paring off the lower margin of the initial channel as sparingly as possible. Bark consumption was thus kept to a minimum, but still not quite as low as in tapping *Hevea braziliensis.* Tapping continued daily without interruption for a period of twelve weeks.

In table 1 are shown the yields for the twelve-week period together with tree girth, bark thickness, initial number of rows of latex vessels, depth of tapping, number of rows of latex tubes severed and remaining, and the amount of bark consumed in vertical inches. All chicle samples were analyzed for moisture content, so that the term "bone-dry chicle" used in this table

FIG. 3. Tree 5 at the end of the twelfth week of tapping. FIG. 4. Tree 5 showing the position of test incisions A, B, and C in relation to the ibidem panel.

means complete lack of moisture. At the conclusion of tapping a chip of bark with the cambium attached was removed at the upper and lower limits of the ibidem panels as well as directly from the tapped areas. From cross sections of these bark samples the initial thickness of the bark, total initial number of latex tubes, and the number severed were counted and computed for each tree. The thickness of the bark shown in table 1 includes only the cortex between the cambium and phellogen. The outer hard corky layers are omitted in these measurements, since they contain only dead and degenerating latex vessels. The number of rows of latex tubes and the thickness of the bark, however, are not constant throughout the length of the bole but vary considerably in different areas to much the same degree as

Haigh (1928) and others have reported for *Hevea braziliensis*. Within limited areas such as the short ibidem panels, the variations are perhaps less marked. Furthermore, the depth of tapping and consequently the number of rows of latex tubes remaining vary considerably in different parts of the tapped panels. Hence, the figures given in table 1 may not be absolutely constant for the entire panels. They relate only to the particular areas from which the bark samples were taken.

TABLE 1

Girth, thickness of bark, rows of latex vessels, depth of tapping, bark consumption, and yield from 12 trees of Achras zapota tapped by the ibidem method daily for 12 successive weeks

Tree No.	Girth (cm.)	Thickness of bark (cm.)	Initial no. of rows of latex tubes	Depth of bark remaining after tapping (cm.)	No. of rows of latex tubes removed in tapping	No. of rows of latex tubes remaining in untapped bark	Bark consumption in vertical cm.	Yield in grams of bone-dry chicle		
								1st day	2nd day	3rd day to end of 12th week
2	80	1.10	30	.35–.40	20–19	10–11	18.12	15.65	0	0
3	73.12	.57	17	.18–.22	10– 9	7– 8	17.50	9.92	1.42	0
4	58.75	.64	15	.22–.29	12	3	16.87	2.32	0	0
5	60.12	.60	21	.20–.22	16–15	5– 6	16.25	10.64	0	0
6	76.25	.95	24	.25–.35	13–12	11–12	18.12	5.46	.4295	0
7	78.75	.68	27	.15–.20	24–20	3– 4	16.25	9.65	0	0
8	67.50	.69	21	.18	19	2	21.87	5.70	0	0
9	65.62	.65	11	.15–.19	10– 9	1– 2	17.17	9.75	.394	0
10	81.35	.80	21	.25–.35	11	10	19.67	13.05	0	0
11	67.50	.69	21	.20–.30	15–13	6– 8	17.80	6.69	0	0
12	56.35	.55	14	.10–.15	12–11	2– 3	15.62	2.80	0	0
13	73.85	.95	26	.20–.39	18–16	8–10	35.00	10.65	0	0

The results shown in table 1 are conclusive and leave little doubt about the lack of response of *Achras zapota* to the stimulus of daily wounding by the ibidem method of tapping. The yield for the first day is higher in proportion to tree girth than in some reported trees of *Hevea braziliensis* and considerably greater than in *Couma guatemalensis* (Karling 1935). With the exception of trees 3, 6, and 9, however, no latex or gum whatever was secured after the initial tapping throughout the twelve-weeks period although as much as eight vertical inches of bark were pared away in some of the trees. The second-day yield in trees 3, 6, and 9 is probably the result of accidental deeper tapping on that day whereby virgin, uncut latex tubes were severed. The latex system of all trees was continuous to some degree, since in no instances were the deeper-lying latex vessels and cambium severed. As is shown in columns 5 and 7 of table 1, an average of 2 mm of bark and six rows of latex tubes were untouched and intact in each tree at

the conclusion of tapping. As soon as it became evident that no further yield could be secured by the ibidem method of tapping after the first day or two an attempt was made to determine the distance above and below to which the initial incisions had drained the latex supply. Accordingly, in tree 1 (fig. 2) the bark was deliberately pared away more rapidly, so that by the end of twelve weeks fourteen vertical inches had been removed. No latex appeared in the daily incisions until ten vertical inches had been pared away. From then on the amount increased until it was sufficient to cover the lower margin of the oblique cut and flow part of the distance down the vertical groove to the collecting cup.

The results obtained from tree 1 indicate that an incision in the bark of *Achras zapota* drains only a limited area even when part of the laticiferous system is intact and that little or no latex can be secured regardless of the number of incisions which may be subsequently made within such a drainage area. Further proof of this was secured from additional experiments on other trees. At the end of the twelfth week (fig. 3) trees 2–13 were tapped in the same manner with a gouge half-way around the bole 16 inches above and 16 inches below as well as directly opposite the initial incision of the ibidem panel, as is shown in figure 4. These three additional channels are designated as A, B, and C in figure 4 and table 2. As is shown in this figure the latex

TABLE 2

Comparative yields (in grams) of the ibidem panels and channels A, B, and C, "16" above, below, and directly opposite the initial incision

Tree No.	Yields			
	Initial cut	Channel A	Channel B	Channel C
2	15.65	..	6.68	39.50
3	9.92	. ..	7.02	13.55
5	10.64	.. ...	12.88	13.95
6	5.46	... .	8.25	13.53
7	9.65	6.43	7.07	11.47
8	5.70	4.49	3.60	9.65
9	9.75	... .	6.37	7.80
10	13.05	8.26	16.85	26.60
11	6.69	....	3.92	6.72
12	2.80	....	8.68	4.48
13	10.65	3.31	4.77	18.55
Average	8.99	5.62	7.78	14.98

flowed quite freely from these channels, but the yields vary considerably. In all but four of the trees the ibidem panel had progressed so close to the ground by the end of twelve weeks that it was impossible to make channel A at an interval of 16 inches. In the four trees from which the data are complete, the yields from channel A are less than those of the initial incisions of the ibidem panel. Likewise, with the exception of trees 5, 6, 10, and 12,

channel B gave less chicle than the initial one. This suggests that the ibidem panel depleted the latex supply for a considerable distance above and below but not appreciably in a lateral direction. The slightly higher yield from channel C in all but one instance might perhaps be regarded as a response to the stimulus of wounding, but in the light of the wide variations in yield from opposite sides of the same tree which have been secured from other wound stimulus experiments, this seems highly questionable. The general averages in table 2 show that the yield from channels A and B are not very far below those of the ibidem panels. The fact that they yielded any chicle at all, while the ibidem incisions remained almost completely dry after the first day clearly indicates that an incision in the cortex of *Achras zapota* which leaves the laticiferous system intact and continuous to some degree, nonetheless, drains only a limited area. This fact is supported by extensive data from other tapping experiments.

The data from this ibidem tapping experiment confirm the results of the preliminary tests and show without much doubt that the sapodilla tree in British Honduras does not respond to the stimulus of wounding as does *Hevea braziliensis*. These results, however, do not prove conclusively the complete lack or absence of response to injury in *Achras zapota*, but only that the method of stimulation employed on the rubber tree does not produce similar results on the chicle tree. While the many diverse methods of tapping which have been tried up to the present time have failed to show evidence of a wound response, it is not improbable, in light of the positive data from *Pinus*, *Hevea*, and other genera, that this characteristic exists to a lesser degree in the sapodilla tree also and that a type of bleeding may possibly be found which will reveal its presence. Nevertheless, it is quite obvious from the above experiment that *Achras zapota* cannot be tapped profitably daily on a limited panel of cortex.

Whether or not the difference in response between *Hevea braziliensis* and *Achras zapota* is entirely due to differences in type, structure, and continuity of the latex system is not certain. The latex tubes of the former species are more continuous, closely anastomosed, and numerous than in *Achras zapota* (Karling 1929), and this difference may be a contributing factor.

SUMMARY

Tapping experiments on *Achras zapota* in British Honduras involving application of the ibidem method commonly used on *Hevea braziliensis* indicate a lack of response to the stimulus of wounding. The initial oblique incisions in the cortex drain the latex from an area approximately 10 to 14 inches above and below, and daily pairings of the lower margin of the incisions within such areas yield no additional latex. The results obtained show that *Achras zapota* cannot be tapped profitably by the ibidem method

and that the successive incisions in the cortex must be spaced approximately 14 to 16 inches apart to secure the maximum yield per tapping.

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PLANT NUTRITION AND THE HYDROGEN ION—III. SOIL CALCIUM AND THE OXALATE CONTENT OF SPINACH

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With the fuller recognition of the fact that the increase of hydrogen on the exchange complex, or colloidal fraction, of the soil is the approximate reciprocal of the loss therefrom of plant nutrient cations, the wide range in soil acidity should augur a wide range in the chemical composition of plants. This range may be expected both between plant species and within the same species. Some species reflect the soil nutrient shortage by reduced growth. Others on the same soil do not manifest lowered mass production as the reflection of the soil fertility shortage. They must therefore have lowered concentrations or totals relative to those soil-given nutrients that are deficient for the other plants.

Among the garden vegetables, spinach is a crop that usually makes ample growth over a wide range of soil differences. Its composition in ash constituents is widely variable (6, 2). Its content of oxalate has also been called to attention for its wide fluctuation (5). Because the oxalate is commonly considered a metabolic by-product, the variable amounts of this carbon compound in the crop as related to different levels of soil calcium and to different degrees of soil acidity may shed some light on the interrelations of all these in the plant processes. The dietary importance of spinach as a carrier of calcium, magnesium, and other nutrients, subject to fluctuations caused possibly by soil variations, encouraged the following study of the oxalate content of spinach in relation particularly to the exchangeable calcium in the soil.

PLAN OF THE EXPERIMENT

As a means of controlling the nutrient supply in the substrate, the subsoil of the Putnam silt loam with its high content of colloidal clay was used as a carrier of the exchangeable nutrients. Since not only the clay fraction (1) but also the silt fraction of this soil type are almost inert toward plant growth (3), the coarser fractions were not removed from the finer colloidal ones. The subsoil clay used had an exchange capacity of roughly 28 M.E. per 100 gms. Twelve of the 28 M.E. were hydrogen, twelve were calcium, and

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the balance of the clay's capacity was taken by other elements in minor amounts.

A series of different amounts of calcium was provided by putting specific amounts of it on the clay and using more or less of the treated clay so as to offer 0, 3, 6, 9, and 12 M.E. per plant. The other nutrients similarly supplied in constant amounts included: nitrogen 10 M.E., potassium and phosphate 6 M.E. each, and 3 M.E. each of magnesium and sulfate per plant.

One series was made up by adding oxides and hydroxides of the elements to the acid colloidal clay to give a nearly neutral condition with a final pH of 6.8. The second series was made up by adding them as salts, namely chlorides and sulfates, so that an acid soil at pH 5.2 resulted. These series will be spoken of as the "neutral" and the "acid" series respectively. The clay carrying its supply of exchangeable ions was mixed with quartz sand; put into porous clay pots; planted to a final crop of one plant per pot; and replicated as 40 pots in a single treatment. The amounts of nutrients and of clay per plant were those given in table 1. The plants were grown from February 10 to April 15, or a period of 64 days, after which the tops were harvested; dried at 65° C.; weighed; and prepared for chemical analyses.

TABLE 1

Nutrients added to the soil to provide variable calcium levels in "acid" and "neutral" soils

	Nutrients per plant							Clay per plant	Resulting pH
	Ca	N	P	K	Mg	S	Cl		
	M.E.	*M.E.*	*M.E.*	*M.E.*	*M.E.*	*M.E.*	*M.E.*	*gms.*	
"Acid" pH 5.2	0	6	6	6	3	3	2	91.5	5.2
	3	6	6	6	3	3	1	100.0	5.2
	6	6	6	6	3	3	2	125.0	5.2
	9	6	6	6	3	5	3	150.0	5.2
	12	6	6	6	3	9	4	175.0	5.2
"Neutral" pH 6.8	0	6	6	6	3	3	....	125.0	6.8
	3	6	6	6	3	3	....	150.0	6.8
	6	6	6	6	3	3	....	175.0	6.8
	9	6	6	6	3	3	....	200.0	6.8
	12	6	6	6	3	3	....	225.0	6.8

RESULTS

The growth of the crop as related to increments of exchangeable calcium on the two soils of different degrees of acidity is shown in figure 1. The neutral soil produced slightly more spinach than the acid soil, a mean increase amounting to 7.8 per cent. In its response to calcium, this crop demonstrated the beneficial influence of the increasing amounts of calcium applied in the exchangeable form, regardless of whether the soil was at pH 5.2 or 6.8.

The chemical analyses included determinations of the oxalate, and of calcium, magnesium, strontium, manganese, and potassium. Some of these determinations were made by regular laboratory methods recognized as standard procedures, while some were spectrographic analyses. The results of these chemical analyses, in terms of concentrations, are assembled in table 2. They are given in terms of totals in the crop in table 3.

TABLE 2

Concentrations (percentage) in the crop of spinach when grown at pH 5.2 and at pH 6.8 with variable amounts of exchangeable calcium offered

Soil pH	Calcium offered	Determinations of					
		Oxalate	Calcium	Magnesium	Strontium	Manganese	Potassium
	M.E.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
	0	4.87	0.92	0.78	0.0044	0.086	6.54
	3	4.54	0.97	0.78	0.0046	0.093	6.86
5.2	6	5.36	1.21	0.91	0.0053	0.093	6.22
	9	5.31	1.23	1.03	0.0064	0.130	6.28
	12	5.01	1.39	1.21	0.0077	0.225	6.49
	0	3.43	0.53	0.52	0.0020	0.043	5.37
	3	3.98	0.60	0.61	0.0023	0.038	5.88
6.8	6	3.73	0.57	0.57	0.0025	0.038	5.86
	9	4.02	0.59	0.54	0.0025	0.040	6.49
	12	3.35	0.66	0.63	0.0026	0.037	6.92

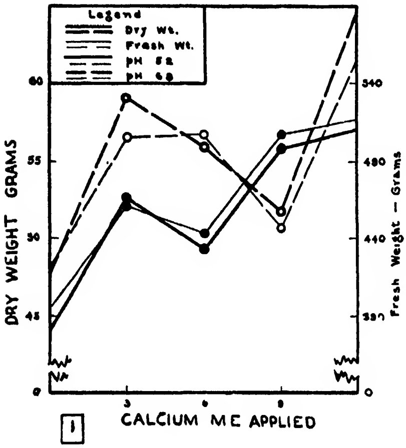
TABLE 3

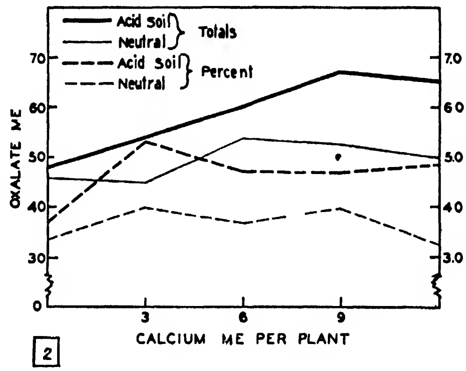
Totals (M.E.) in the crop of spinach when grown at pH 5.2 and at pH 6.8 with variable amounts of exchangeable calcium offered

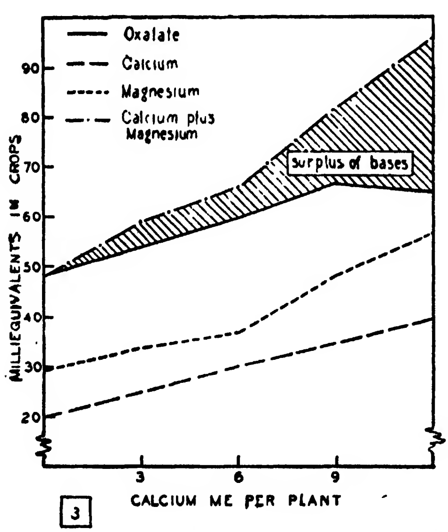
Soil pH	Calcium offered	Determinations of					
		Oxalate	Calcium	Magnesium	Strontium	Manganese	Potassium
	M.E.	*M.E.*	*M.E.*	*M.E.*	*M.E.*	*M.E.*	*M.E.*
	0	48.8	20.3	28.5	0.44	1.38	73.7
	3	54.4	25.7	34.0	0.56	1.80	92.5
5.2	6	60.1	29.8	36.9	0.59	1.68	78.5
	9	67.4	34.5	47.7	0.81	2.66	89.9
	12	65.0	39.7	57.0	1.00	4.67	94.7
	0	37.3	12.8	20.5	0.22	0.75	65.6
	3	54.2	18.1	30.3	0.31	0.84	90.1
6.8	6	47.3	16.1	26.4	0.32	0.78	83.8
	9	47.3	15.2	23.0	0.29	0.75	86.0
	12	48.5	21.2	33.0	0.38	0.87	112.8

One of the outstanding features of the chemical composition of the spinach crop is its higher concentrations and higher total contents of the oxalate,

and of calcium, magnesium, strontium, manganese, and potassium, when grown on the soil originally at pH 5.2 than when on the soil at pH 6.8. In addition, these different concentrations and totals increased, in general, with the increments of calcium in the acid soil, but not so much so on the neutral soil. These increases were apparently associated with increased metabolic performances by the plant as indicated by the larger amounts of oxalate. The







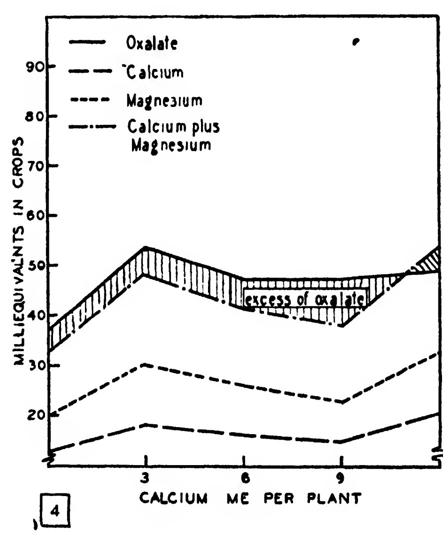


FIG. 1. Weights of spinach (dry and fresh) in relation to the exchangeable calcium applied to the soil at different degrees of acidity. FIG. 2. Concentrations and total amounts of oxalate in spinach as related to the exchangeable calcium in the soil. FIG. 3. Amounts (M.E.) of oxalate, calcium, magnesium, and of the latter two combined, in the spinach crop in relation to the exchangeable calcium in an acid soil, pH 5.2. FIG. 4. Amounts (M.E.) of oxalate, calcium, magnesium, and of the latter two combined, in the spinach crop in relation to the exchangeable calcium in a neutral soil, pH 6.8.

concentrations of the oxalate in the spinach were higher on the acid than on the neutral soil, but not significantly related to the amounts of applied calcium at either pH as is shown in figure 2. That the total amounts of the oxalate in the spinach crop were related to the increments of applied calcium on the acid soil is very evident from the uppermost graph. On the neutral soil the amounts are lower and less closely related to the calcium applications.

Since the oxalate in chemical combination with calcium forms a very insoluble compound, and likewise when combined with magnesium, it will be interesting to note the amounts of oxalate as they are excessive or deficient for complete precipitation of the calcium and magnesium and thus put these two nutrients into possibly inert forms so far as ordinary digestive processes are concerned. These possible relations between the amounts of oxalate in contrast to those of calcium and magnesium combined are best shown in figure 3 for the acid soil and in figure 4 for the neutral soil.

For the acid soil, the combined amounts of calcium and magnesium were increasingly greater than their equivalent in oxalate as more calcium was applied. The calcium treatment may thus be considered as providing both calcium and magnesium in the crop in amounts beyond those which would be precipitated by the oxalate. There would then be available this surplus of these bases, calcium and magnesium, of possible nutritional value even if significant amounts of them had been made insoluble through precipitation by the larger amounts of oxalate. This surplus of bases is relatively larger as more calcium is applied. This is shown by the ratio of the oxalate to the equivalent of calcium, which ratio became narrower, or shifted from 2.4 to 1.6, as more calcium was added to the soil at the outset. It raises the question whether additional offering of calcium might not have given a ratio of one or less, or enough calcium so that it alone might serve to precipitate all the oxalate.

Though the magnesium was applied in constant, exchangeable amount to the soil, the total amounts of it in the crops increased with the increments of calcium applied to the soil. In all cases the magnesium in the crop was larger than the calcium by 25–40 per cent on the acid soil series, and by 50–60 per cent on the neutral soil. But even then in the latter case, or on the neutral soil, the totals of magnesium were less, in general, than those of calcium on the acid soil. Hydrogen ion absence had reduced the calcium intake by the crop more than the intake of magnesium, or the presence of the hydrogen ion activates the calcium for crop use relatively more than it does the magnesium.

The order of these three ions, viz., hydrogen, calcium, and magnesium, in their decreasing degree of adsorption on the clay colloid, may be suggestive. According to this lyotropic series (4) hydrogen is adsorbed to a greater degree than calcium, and calcium to a greater degree than is magnesium.

Accordingly, in the presence of hydrogen to place calcium and magnesium as second and third in the series, the difference in their migrations into the plant is less than when in the absence of hydrogen they are first and second in the adsorption series. Such visualization of these differences may not be the facts, but it is suggestive and helpful in connection with the differences in mobilization of these two bases into the plants in the absence and in the presence of the hydrogen ion on the clay.

In the case of the neutral soil, the combined amounts of calcium and magnesium were not the equivalent of the amounts of oxalate, except where 12 M.E. of calcium were offered per plant. At this high level of calcium offered on the neutral soil, it is important to note that both the oxalate and the calcium in total were just close to the equivalent of that in the crop on the acid soil given no exchangeable calcium. There was then an excess of oxalate which might be expected to precipitate all of the calcium and magnesium and lead us to believe these mineral elements in the crop unavailable for human digestive use. The crop grown under such conditions would not provide these minerals as a vegetable of this type is commonly believed to do.

With the oxalate as one of the plants' metabolic by-products, then, it is evident that the plants were physiologically more active when grown on the acid soil, pH 5.2, than when on the neutral soil, pH 6.8. This is indicated by the higher concentration of oxalate as well as of nutrient cations in the plants on the former than on the latter. This difference in the oxalate amounted as a maximum to 2.0 per cent, and as a minimum to 0.52 per cent, as concentrations in the dry matter. As a mean, the percentage content of oxalate was one-third higher in the former because of the acid condition of the soil. Even though the concentration of oxalate was higher, there was more than enough of the oxalate-precipitating bases taken by the plants from the soil under acid conditions to combine it into the less soluble forms. On the neutral soil, the concentration of oxalate was lower than that in the acid soil by one-fourth, as a mean. But even then, because of the deficiency in the total of the two oxalate-precipitating bases which were less than their equivalent in oxalate, this excess of the oxalate could precipitate and make unavailable some of the calcium contributed to the human digestive mix from other food sources. Thus the spinach might not only be valueless as a calcium contributor, but might even be damaging to the effects by other foods serving this purpose (7).

Whether such views are the actual facts of human digestion, and whether the soil fertility supporting their growth determines the degree to which vegetables are digestive satisfactions or disappointments in our dietary, remains to be more fully determined. They suggest, however, that it might be well to test this hypothesis by means of some assays with smaller animals as help in appreciating the possible situation in human nutrition. Only by

such means can the final facts be fully established even though plant composition differences in relation to fertility variations suggest that human deficiency troubles may find their origin in the deficient calcium and magnesium of the soil on which such vegetables and possibly other products are produced.

SUMMARY

By using the oxalate concentration in the spinach crop as an index of the plants' performances on soils with increasing amounts of calcium under pH of 5.2 and of 6.8, it was shown that the concentration of oxalate in the crop on the acid soil was higher than on the neutral soil. The total in the crop was similarly higher. When the total oxalate was related to the totals of calcium and magnesium it was shown that on the acid soil the amounts of calcium and magnesium taken together were more than the equivalent of the oxalate. With larger additions of calcium to the soil, these two basic cations were present as an increasing surplus over the oxalate.

On the neutral soil the combined amounts of these two cations were not the equivalent of the oxalate except in the case of the soil with the highest calcium application. This soil gave a spinach crop with an excess of the oxalate. Such a result raises the question whether this excess of oxalate may not represent a disturbing condition in the form of calcium deficiency for the plant functions as normal growth.

If the oxalate is considered as removing the two bases, calcium and magnesium, out of reaction in the digestive processes of animals, these results suggest that calcium as a soil treatment for spinach on an acid soil may provide the crop with calcium and magnesium as surpluses over that combined with the oxalate and therefore may contribute some of these minerals of nutritional use. If this soil treatment is applied on a neutral soil, or as a carbonate that should result in neutrality, there is the suggestion that the crop may be so deficient in bases with respect to its oxalate content that this excess of oxalate may not only make the spinach of no nutritional value as a provider of these bases, but may even make it injurious as this excess of oxalate disturbs the calcium coming from other food sources. The composition data of the spinach suggest the need for attention to both the calcium and the magnesium supplies, and to the reaction of the soil growing this crop if the fullest values in terms of its content of these nutrient bases are to be realized.

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THIAMINE PRODUCTION BY ACTINOMYCES VIRIDOCHROMOGENUS

J. ARTHUR HERRICK AND CONST. J. ALEXOPOULOS[1]

During the course of some recent studies on the inhibitory effects of actinomycetes on fungi, six species of *Actinomyces* were grown on liquid media containing sucrose, peptone, and inorganic salts. The mycelium was removed by filtration, the filtrates acidified and then sterilized by autoclaving. Portions of the filtrate from each *Actinomyces* were inoculated with each of nine species of fungi. The degree of inhibition was to be determined by comparing the amounts of fungous mycelium produced in the filtrates with that produced in suitable controls. Owing to the uncertain effects of autoclaving on the inhibitory substances this technique was abandoned for the time being, in favor of growing the actinomycetes and the fungi in apposition on agar (2). Observations made while the filtrate experiments were still in progress, however, revealed that the growth of *Stereum gausapatum* was considerably greater on the filtrate from *Actinomyces viridochromogenus* than on the control medium. The stimulatory effect of this filtrate on *S. gausapatum* was strikingly similar to the effect produced by thiamine on the same fungus (6). The possibility that *A. viridochromogenus* synthesizes and liberates thiamine in culture seemed likely. The present paper deals with the investigation of this problem.

EXPERIMENTAL STUDIES

A liquid culture medium was prepared according to the following formula:

Sucrose	30.0 gm.
Asparagine	1.5 gm.
$MgSO_4$	0.5 gm.
K_2HPO_4	1.0 gm.
KCl	0.5 gm.
$FeCl_3$	0.1 gm.
Distilled water	1.0 liter

The H-ion concentration was adjusted to pH 9 and the medium autoclaved for 10 minutes at 15 lbs. pressure. This caused a precipitate to form

[1] The writers wish to express their appreciation to Dr. H. A. Cunningham, Chairman, Department of Biology, Kent State University, for his encouragement and cooperation in providing research facilities.

which was removed by filtration through filter paper. The medium was then restored to its original volume by the addition of distilled water and the H-ion concentration adjusted to pH 7.3. Several 200-cc. Erlenmeyer flasks, each containing 50 cc. of the medium, were sterilized by autoclaving for 15 minutes at 15 lbs. pressure. One of the sterile flasks was opened and the pH of the medium was found to be still 7.3. One-half of the flasks were then inoculated with a heavy spore suspension of *A. viridochromogenus;* the remaining flasks were reserved to be used later as controls. After 27 days, the *Actinomyces* mycelium was removed by filtration through filter paper, the filtrate restored to its original volume with distilled water, and the acidity adjusted to pH 5.4. The medium was returned to its original flasks in the same amounts as before. The sterile control medium was similarly restored to volume and its pH adjusted to 5.4. The flasks of media were again sterilized by autoclaving for 15 minutes at 15 lbs. pressure. On the following day the flasks of media were inoculated as follows: a) three flasks of *Actinomyces* filtrate and three flasks of control medium were inoculated with *S. gausapatum,* b) three flasks of *Actinomyces* filtrate and three flasks of control medium were inoculated with *Phycomyces blakesleeanus.*

S. gausapatum produced only a scanty submerged growth on the control medium, whereas it produced a heavy surface mycelium on the *Actinomyces* filtrate. The difference between the growth of this fungus on the *Actinomyces* filtrate and on the control medium was even more striking than before because in the inhibition experiments a peptone medium was employed which supports the growth of *S. gausapatum* much better than does the asparagine medium (5).

Within three days after inoculation, *P. blakesleeanus,* which is a rapidly growing fungus, had produced a heavy surface mycelium and an abundance of sporangiophores in the flasks of *Actinomyces* filtrate. In the control medium, there was a weakly developed, submerged mycelium and absolutely no sporangiophores. A week after inoculation, the three cultures of *Phycomyces,* in each of the two media, were filtered through two previously dried and weighed filter papers, respectively, and the dry weight of the mycelium determined. The dry weight of the mycelium of *P. blakesleeanus* produced in three flasks of *Actinomyces* filtrate was 137 mgms., while that produced in three flasks of control medium was 87 mgms.

As a further check, a solid medium, made by adding 2 per cent of "Difco bacto-agar" to the above formula, was prepared and poured into Petri dishes. The plates were inoculated with *A. viridochromogenus* along a two-inch line near one edge of the plate. One week later the same plates were inoculated near the opposite edge with *P. blakesleeanus.* A sparse growth of *Phycomyces* covered the plate in a few days. Near the line of *Actinomyces* growth

there appeared a much more dense mycelium of *Phycomyces* as well as an abundance of sporangiophores.

DISCUSSION AND CONCLUSIONS

It is a recognized fact that *Phycomyces blakesleeanus* cannot grow well without thiamine or its immediate precursors, and further, that the quantity of mycelium produced may be determined by the amount of thiamine available (1). The only thiamine in the original liquid culture medium was the slight trace which is believed to occur as an impurity in the asparagine (3, 8). In the solid medium employed in these experiments there are two sources of thiamine, namely, that provided by the asparagine, and that which occurs in the "Difco bacto-agar" used as a solidifying agent (4). The pronounced growth and sporangiophore production by *P. blakesleeanus* in the medium which had previously supported *A. viridochromogenus*, in contrast to the poor growth and absence of sporangiophores in the control medium, is offered as proof that *A. viridochromogenus* produces significant amounts of thiamine, or its immediate precursors, in culture.

It is of interest to note that Mackinnon (7) has recently demonstrated that *Actinomyces albus* is also able to produce thiamine in culture media. Further investigations are now in progress to determine whether or not thiamine production is a general property of the genus *Actinomyces*.

SUMMARY

Actinomyces viridochromogenus was grown on an alkaline liquid medium. The cultures were then filtered, the filtrate acidified and autoclaved. This filtrate was found to support a heavier growth of *Stereum gausapatum* than similar media which had not previously supported *A. viridochromogenus*. Similar, but more pronounced results were obtained when *Phycomyces blakesleeanus* was substituted for the *Stereum*. When *P. blakesleeanus* was grown on agar plates previously inoculated with *A. viridochromogenus*, *Phycomyces* produced a much denser mycelium and a crop of sporangiophores in the vicinity of the *Actinomyces* colony. Since the growth of the *P. blakesleeanus* is a recognized assay for thiamine it is concluded that *A. viridochromogenus* produces thiamine in culture.

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AN ANALYSIS OF THE WOOD OF THE THREE COMMERCIAL SPECIES OF WHITE PINE

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In the course of work on the lumber of the three white pines of commerce —*Pinus strobus*, northern white pine, *P. monticola*, western or Idaho white pine, and *P. lambertiana*, sugar pine—it was necessary to distinguish microscopically between the wood of these species. An examination of the literature failed to disclose any very definite and infallible criteria which could be used, and therefore a brief treatment of the results of investigations on the microscopic characters of the wood is herewith presented, together with some broader considerations of the interrelationships of these species.

The similarity in the wood of these pines is almost to be expected, since the trees have so much in common; in fact, the western white pine has been considered a variety of the northern. All of them are tall and straight five-needled pines, having needles of nearly the same length, and similar cones. The latter are long-stalked, comparatively elongated, and rather thin-scaled, the scales are unarmed, and the seeds have relatively large wings; the seeds of all of them mature in the second season. *Pinus strobus* is usually distinguished from the others by its more delicate and slightly longer leaves and by its somewhat shorter cones, while *P. lambertiana* differs from *P. monticola* especially in having larger cones and slightly coarser needles. In internal leaf structure, *P. strobus* and *P. monticola* are similar in having thinner-walled subepidermal cells, while *P. lambertiana* has thicker-walled cells beneath the epidermis and usually around the resin ducts (Coulter and Rose 1886; Sargent 1897). Although there are constant differences between these species, they are fundamentally similar and obviously related. *Pinus strobus* covers a wide area in eastern North America; *P. monticola* is found in northern Montana and Idaho and farther west from British Columbia into California; *P. lambertiana* extends from Oregon southward into Lower California (Sargent 1897, 1933; Britton 1908).

On the basis of wood characters, Record (1934) groups *Pinus strobus* and *P. monticola* together in his key and states that the lumber of the latter is "ordinarily not distinguishable" from that of the former. The essential similarity of the wood of these three species is also evident from a study of the table of characters given in the article by Phillips (1941). In dealing with minute features, Brown and Panshin (1940), in their excellent book, separate these three pines on the tangential diameter of the longitudinal resin canals and on the number and general character of the pits leading

from the ray parenchyma cells to the spring tracheids. They characterize the ray parenchyma pits of *P. lambertiana* as "large (window-like)," "rounded and more or less widely spaced"; those of *P. monticola* are described as "large (window-like)," "more or less angled and occupying most of the back wall"; in *P. strobus* they are called "large (window-like)," "more or less angled and occupying most of the back wall." Penhallow (1907) describes the ray parenchyma pits of *P. lambertiana* as "very large, oval"; those of *P. monticola* as "large, oval or oblong or lenticular"; those of *P. strobus* as "very large, oval, or lenticular." Larsen and Woodbury (1916) have also made careful comparisons of *P. strobus*, *P. monticola*, and *P. lambertiana*. They caution judiciously that identification of the wood by means of either gross or minute characters should rest on comparison of all available features, and not on any one alone. Anatomical differences between groups of species of pines are given by Rol (1932); using the details of the ray tracheids and of the ray parenchyma pits, he places *P. strobus* and *P. monticola* both in the section *Strobus*, without listing further distinguishing features. *P. lambertiana* is not included in his scheme. The general wood anatomy of the pine, especially of *P. strobus*, is taken up by Jeffrey (1917). Spaulding and Fernow (1899) have considered *P. strobus* in detail. General wood characters of all the commercial pines are thoroughly discussed by Hall and Maxwell (1911).

The gross characteristics of the wood of these three pines show striking similarities. In each species the wood is rather soft, straight-grained, fairly light in color and in weight, and the transition from spring to summer wood is gradual. The resin canals extend in both longitudinal and transverse directions, and they are numerous and easily visible to the naked eye. Sugar pine is distinguished from the other two species, macroscopically, by its somewhat coarser texture, its more conspicuous and often dark brown resin ducts, by the sugary exudation often found in newly cut wood, by its stronger odor, and by its slightly different color. Idaho white and northern white pine wood vary in color from nearly pure white to reddish brown; sugar pine is not so nearly white in color, nor is it ever quite so reddish brown as the other two. Idaho white pine wood can hardly be distinguished from that of the northern white pine on gross characters, though the color is often somewhat lighter in the former.

The more minute features of the wood of these three pines, as revealed by microscopic examination, are also similar. The general characters of the tracheids, of the resin ducts, the absence of dentate ray tracheids, and the general similarities of the pits of the ray parenchyma cells, as contrasted with those of other species of pine—all show a striking likeness in these species. However, Brown and Panshin (1940) as well as Larsen and Woodbury (1916) point out that the diameter of the resin ducts is greatest in

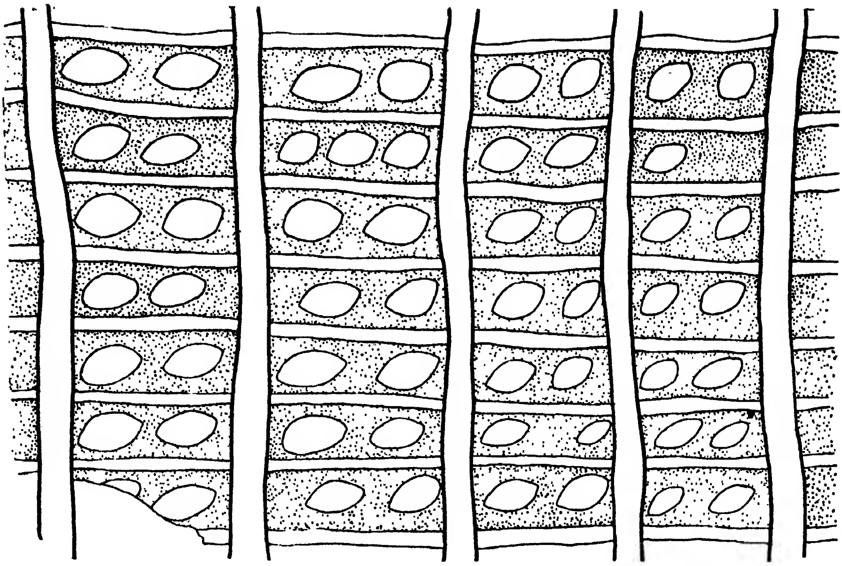
P. lambertiana and least in *P. strobus,* and they also indicate, in somewhat general terms, that there are differences in the pits on the radial walls of the pith-ray cells. Brown and Panshin further state that the average diameter of the tracheids is greatest in *P. lambertiana* and least in *P. strobus,* though in this respect the difference between *P. lambertiana* and *P. monticola* is slight.

In the present study mature wood and sections of wood obtained from four different sources were used for comparison. The results are based on large numbers of sections. They do not include investigations of twigs, of the earliest annual rings, of roots, or of trees growing under unusual conditions.

A microscopic study of these three species shows that one of the most reliable differences in minute structure is found in the pits of the ray parenchyma cells. These pits, which occur on the walls of the ray parenchyma cells in contact with the radial walls of the tracheids, vary in size, in shape, and in distribution. Figures 1, 2, and 3 are radial sections of the wood of *Pinus lambertiana, P. monticola,* and *P. strobus,* respectively. The vertical lines represent sections longitudinally through the tracheids, while the horizontal lines show the ray parenchyma cells. A ray tracheid is also shown in figure 2. All the drawings were made from spring tracheids.

It is obvious from figure 1 that each ray parenchyma cell in *P. lambertiana* usually has two pits per tracheid. However, although this is rather generally true, there may be only one, as frequently happens also in the narrow tracheids of the summer wood, or there may be three or occasionally four in wide tracheids of the spring wood. Brown and Panshin and Larsen and Woodbury speak of these pits as round, or rounded, but this description seems hardly adequate. In the spring wood they are somewhat lemon-shaped, often more or less apiculate both above and below, along a diagonal axis, as shown in figure 1. Although this shape of the pits is not invariable, it very generally occurs, and it seems to be the most reliable microscopic character in identification of the species. These pits are somewhat smaller, both actually and relatively, than in the other two species. They are also more uniform in size and more regular in outline than in *P. monticola* and *P. strobus.* In the summer wood they are distinctly narrowed along the diagonal axis.

From the standpoint of minute wood anatomy, the structure of *P. monticola* comes close to that of *P. lambertiana.* Here again there are usually two pits per ray parenchyma cell for each tracheid, but there may be just one, especially on the narrower tracheids, or there may be more than two, though usually there are not more than three. In this species the pits vary greatly, as is shown in figure 2. Some of them are strongly suggestive of those characteristic of *P. lambertiana,* though usually they lack the suggestion of the apiculate feature found in the latter species. Many of the pits of *P. mon-*



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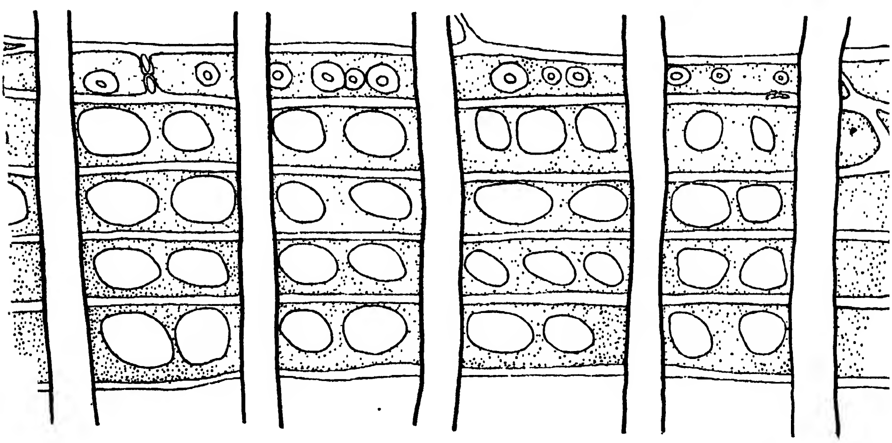

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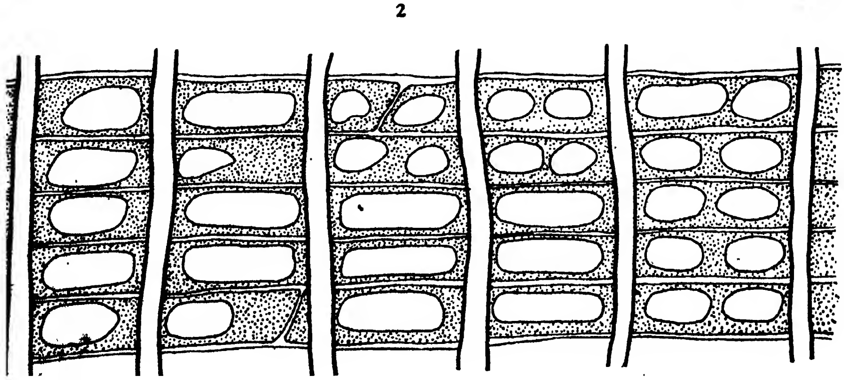

ticola are rounded, though usually somewhat irregularly so, and some of them are distinctly oval. If there is just one pit per tracheid, it may be distinctly flattened horizontally and rounded at the ends. The pits of *P. monticola* normally occupy more of the available wall space than those of *P. lambertiana,* and they are somewhat larger; they lack the uniformity of orientation so characteristic of the latter species.

In the wood of the northern white pine, *P. strobus,* the diameter of the tracheids of the spring wood is normally less than in the other two species.

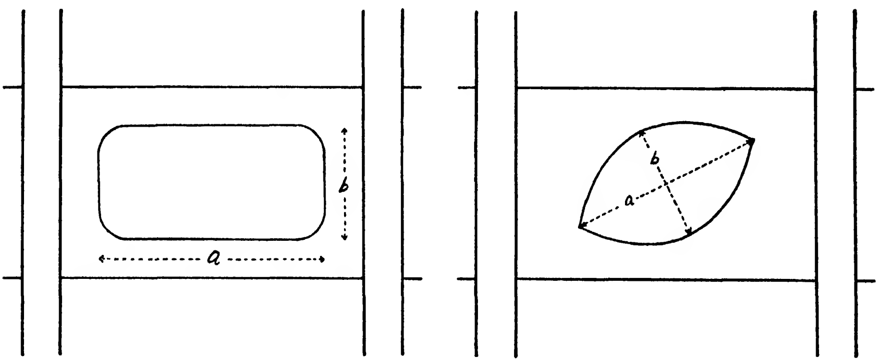

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FIG. 4. Diagram of an oblong ray parenchyma pit. FIG. 5. Diagram of an elliptical ray parenchyma pit. The axes that were measured for tables 1 and 2 are indicated by the dotted lines, *a* and *b*.

Often there is a single large oblong pit for each ray parenchyma cell per tracheid, as shown in figure 3. Not uncommonly there are two such pits, however, and occasionally there may be three. If there are two or three pits, they are not so elongated, but more rounded; the suggestion of an apiculate feature is rarely present. These pits are larger than those of the other two species, and they occupy a greater proportion of the available wall space. On the basis of these pit characters, *P. strobus* is distinguished from the others.

Data to illustrate these features are given in tables 1, 2, and 3. The pit measurements of table 1 were made with an ocular micrometer. The length of elliptical pits was taken along the long axis, as indicated by *a* in figure 5, regardless of whether this axis was horizontal or oblique; the short dimen-

Explanation of figures 1–3

FIG. 1. Longitudinal section of the spring wood of the sugar pine, *Pinus lambertiana,* showing the ray parenchyma pits. The pits here are lemon-shaped, some more or less apiculate, usually two per ray crossing. × 370. FIG. 2. Longitudinal section of the spring wood of the Idaho white pine, *P. monticola,* showing the ray parenchyma pits. The pits here are mostly somewhat irregularly rounded or slightly oblong, usually two per ray crossing. A ray tracheid is shown above. × 370. FIG. 3. Longitudinal section of the spring wood of the northern white pine, *P. strobus,* showing the ray parenchyma pits. The pits here are mostly oblong, often one per ray crossing. × 370. Pits in the upper and lower walls of the ray parenchyma cells are not shown in these illustrations.

sion, *b*, was taken at right angles to the long one. Oblong and rectangular pits were measured along the horizontal and vertical axes. The pits were chosen at random. These measurements are significant as indicating relative rather than actual dimensions. It would be possible, for instance, to choose pits of *P. lambertiana* with dimensions larger than those of chosen pits of *P. strobus*. In no case were measurements made from summer tracheids.

An inspection of table 1 indicates that the pits are longest in *P. strobus* and

TABLE 1

Dimensions of ray parenchyma pits on radial walls of tracheids

	P. lambertiana Average in μ per pit		*P. monticola* Average in μ per pit		*P. strobus* Average in μ per pit	
	Length	Width	Length	Width	Length	Width
2 pits per ray crossing	17.5	12.0	15.1	15.0	19.0	15.9
	(200 pits)		(300 pits)		(200 pits)	
1 pit per ray crossing	21.7	13.6	21.5	15.0	31.2	14.5
	(200 pits)		(300 pits)		(200 pits)	
Totals	19.6	12.8	18.3	15.0	25.1	15.2
	(400 pits)		(600 pits)		(400 pits)	

narrowest in *P. lambertiana*. This is true when there are two pits per ray crossing, and also when there is only one. *P. monticola* is more or less intermediate. The average length and width of pits of *P. monticola* are practically equal when there are two per ray crossing.

In order to analyze the character of these pits further, the length and width of two hundred of each species were measured, and a record was kept of the approximate shape of each of these—i.e., whether they were oblong, circular, or elliptical. The area of each of the oblong pits was calculated by multiplying length by width; the area of the circular ones was calculated from the formula πr^2; and the area of the elliptical ones was calculated from the formula of the area of an ellipse, πab. These results were then assembled, and the average area per pit, in sq. μ, is given in table 2. In area, as well as

TABLE 2

Area of ray parenchyma pits on radial walls of tracheids

	P. lambertiana Average area in sq. μ per pit	*P. monticola* Average area in sq. μ per pit	*P. strobus* Average area in sq. μ per pit
2 pits per ray crossing	165.4 (100 pits)	176.4 (100 pits)	276.2 (100 pits)
1 pit per ray crossing	269.0 (100 pits)	278.7 (100 pits)	448.8 (100 pits)
Totals	217.2 (200 pits)	227.6 (200 pits)	362.5 (200 pits)

in linear dimensions, the pits of *P. strobus* are the greatest, those of *P. lambertiana* are the least, while those of *P. monticola* are intermediate.

Next, 2,175 pits of these three species were examined with a view to determining how many of each were elliptical, how many circular, and how many oblong or rectangular. The results are summarized in table 3. It is evident

TABLE 3

Shape of ray parenchyma pits on radial walls of tracheids

	2 pits per ray crossing (442 or 61.9%)		*P. lambertiana* 1 pit per ray crossing (272 or 38.1%)		Total (714)	
Oblong	63	14.3%	115	42.3%	178	24.9%
Circular	37	8.4%	0	0.0%	37	5.2%
Elliptical	342	77.4%	157	57.7%	499	69.9%
	2 pits per ray crossing (494 or 67.3%)		*P. monticola* 1 pit per ray crossing (240 or 32.7%)		Total (734)	
Oblong (and square)	314	63.6%	208	86.7%	522	71.1%
Circular	75	15.2%	6	2.5%	81	11.0%
Elliptical	105	21.2%	26	10.8%	131	17.9%
	2 pits per ray crossing (450 or 61.9%)		*P. strobus* 1 pit per ray crossing (277 or 38.1%)		Total (727)	
Oblong	293	65.1%	271	97.8%	564	77.6%
Circular	45	10.0%	1	0.4%	46	6.3%
Elliptical	112	24.9%	5	1.8%	117	16.1%

that those of *P. lambertiana* are more commonly elliptical than any other shape, while those of *P. strobus* are usually oblong, and *P. monticola* again is intermediate. Many of the pits of this last species, especially when there were two per ray crossing, were nearly or quite square. Many of the elliptical pits, particularly of *P. strobus,* were not diagonally oriented, and they gave no suggestion of the apiculate feature.

Comparing the parenchyma pit characters of *P. lambertiana* and *P. strobus,* it is obvious that there are distinct differences, as has been pointed out. Microscopically these two species, though fundamentally similar, could hardly be confused. It would be difficult to conceive of a more perfect series of intergradations in pit characters between these two species than is offered by *P. monticola.* In this last species some of the pits suggest *P. lambertiana* and some suggest *P. strobus.* However, the majority of the pits are not the

same as in either of those two species, but are intermediate in character between them. It is thus usually possible to distinguish *P. monticola* by the use of these microscopic wood characters. The admonition of previous writers that all the microscopic characters ought to be used for identification should be emphasized again; numerous sections should be studied, since different parts of the same wood show considerable variation, even in small pieces.

These microscopic distinctions are not merely biological and academic, since the lumber also shows differences in standard tests. According to Markwardt and Wilson (1935), the wood of *P. monticola* is the "strongest" in certain ways, though this tree is normally the smallest of the three.

On the basis of external leaf characters, *P. lambertiana* and *P. monticola* obviously are more closely similar to each other than to *P. strobus*. The gross cone features also form a series, with *P. lambertiana* and *P. strobus* at either end. Macroscopically, the woods of *P. strobus* and *P. monticola* are hardly distinguishable from each other, though they can be told, generally, from that of *P. lambertiana*. The microscopic wood characters, on the other hand, form a beautiful series with *P. strobus* distinct at one end, and *P. lambertiana*, also distinct, at the other. The wood of *P. monticola*, as shown above, contains an almost imperceptible series of intergradations microscopically between the two; possibly it is a bit closer to *P. lambertiana* than to *P. strobus*.

The distribution of the species fits in well with the microscopic features of the wood, for *P. strobus* is found in eastern North America, *P. lambertiana* in the far west, and *P. monticola* somewhat between, although partially overlapping the range of *P. lambertiana*.

Both Britton (1908) and Sargent (1933) treat these three species in the same sequence—namely, *P. strobus, P. monticola,* and *P. lambertiana*—and that is not just fortuitous. Nuttall (1849) considered *P. monticola* as a variety of *P. strobus*. The microscopic wood characters strongly support the sequence of Britton and Sargent, and they emphasize the fact that *P. monticola* is a species in many ways intermediate between the other two, but distinct nevertheless. This should not be construed, however, as indicating that this is a definite, closed series. There are other related species, both in western North America and in Asia, which probably form a part of this series. A study of these forms is at present under way.

Bailey (1910) has shown how the larger ray parenchyma pits may be derived from smaller ones. Bailey and Faull (1934) have pointed out the variations that occur in the microscopic features of *Sequoia*, and more recently Bannan (1941a, 1941b, 1942) has dwelt on this same subject for a number of conifers, more especially the genera *Thuja* and *Juniperus*, but others as well.

In this connection it should be noted that in the present study mature wood only was examined. An intensive analysis of twigs, of roots, and of injured members might well bring out differences not here recorded. It would be of interest to study these species growing under varied conditions and trying environments. Such work should throw further light upon the interrelationships of these forms.

SUMMARY

The pits of the ray parenchyma cells in contact with the tracheids are described in detail for the three white pines of commerce. As shown in figures 1, 2, and 3, they are large and oblong in *Pinus strobus,* somewhat smaller and lemon-shaped in *P. lambertiana*, and intermediate in *P. monticola.* In ray parenchyma pit characters the last species is a little closer to *P. lambertiana* than to *P. strobus.* In other features, both gross and microscopic, *P. monticola* also is intermediate between the other two.

The series thus formed on the basis of wood characters coincides with that appearing in the manuals and conforms with the distribution of these three species in nature, though other species undoubtedly also form a part of this series.

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CORNUS SERICEA L. (C. STOLONIFERA MICHX.)

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There has been little agreement among the treatments of the small complex of forms centering around what has been called *Cornus stolonifera* Michx. By some workers each discernible element has been considered a separate species. Others have regarded some of the groups as varieties of those which they have termed species.

Enough specimens have been accumulated in the herbaria in Washington, Philadelphia, and New York to enable one to get a fair idea of the diversity and distribution of these forms. An examination of some hundreds of sheets, variously named *Cornus stolonifera, C. baileyi, C. pubescens, C. instolonea, C. californica, C. interior, C. occidentalis, C. nelsoni,* etc., has convinced me that the whole variable population is best regarded as a single species, and that this might not be termed even a collective species by some conservative students. Current treatments seem mostly to be the result of a definite effort to recognize species, no matter how indistinguishable they are.

With the exception of some alleged differences in the shape of the stone in the fruit, the characters which have been used to separate the forms lie in the color of the twigs, the abundance and distribution of two types of hairs and the prominence of the veins on the lower leaf surface. Whether or not the plant is stoloniferous is often mentioned, but this is ordinarily indeterminable in the herbarium, except as rarely noted by the collector.

It is on the basis of the hair types that the different forms are commonly recognized, and fairly easily distinguished. Actually, these differences are so slight and so inconstant that they cannot represent very fundamental genetic changes. A single allelomorphic series of genes might well be responsible for these differences. The geographical distribution of the plants differing by these characters is such as to indicate merely a variation in composition of the local partial populations from place to place in a single large population, giving evidence of some environmental selection.

The differences in the size, shape, and sculpturing of the stone, though conspicuous, cannot be ascribed much importance. There is too much local variation, even within one inflorescence. The only stone character which I consider of any consequence in this species is the presence or absence of sculpturing, usually in the form of longitudinal ridges, on the lateral faces of the stones. This seems to be definitely associated with the plants which have been called *C. californica* and *C. pubescens*.

Fruit color is variable. Usually it is white, but occasionally it is pale blue.

In flower size there seems to be some significant variation. In eastern and northern specimens the range of variation in length of petals is ordinarily 2 to 3 mm. Much of the western material, especially that from California, varies around 4 mm., some even reaching 5 mm.

From the foregoing, it seems that the complex can be considered merely an aggregation of forms, based on the differences in pubescence. Even though some of these are more or less restricted in their distribution, my opinion is that genetically they do not merit the rank of *varietas*. However, on the basis of the larger flowers and the sculptured stone the two Pacific forms may be considered to represent, together, a separate subspecies, here called ssp. *occidentalis*.

The division of the species thus into two subspecies, each with several poorly localized forms, seems to be nearer to a representation of the actual diversity than any other arrangement that I know of. It might well be argued that even so the group is segregated too finely. Certainly the diversity is no greater than that within many species which have never been divided in any way.

This scheme leaves a number of plants in the western part of the range which do not fit either subspecies. It may be assumed that the presence, in western specimens, of any of the following characters—spreading hairs, large flowers, or sculpturing on the faces of the stones—is an indication of some ssp. *occidentalis* heredity. Where these characters are all or mostly present, the plant may be considered to belong to that subspecies. Where a furrowed stone or large flowers are found in eastern specimens, I would suspect past crossing with the closely related *C. amomum* Mill. This apparently happens very rarely.

Unfortunately, in spite of the discussions by Farwell (Rhodora **33**: 68–72, 1931) and Rickett (Rhodora **36**: 269–272, 1934), the correct epithet to be applied to this species has not been settled. Both of the above authors find that the name *Cornus sericea* L. applies, with the exception of the Plukenet reference, to what has been known as *C. stolonifera* Michx. That this is true is obvious from a scrutiny of the original description which is detailed enough. The only discrepancy is in the color of the fruit, which probably came from the Plukenet reference, though there are blue fruited forms of the plant under discussion (cf. *Fernald 305* [US]). In addition to this, both of the above writers refer to the specimen in the Linnaean Herbarium of *C. sericea* as being without doubt what has been called *C. stolonifera* Michx. In Jackson's index to the Linnaean Herbarium this specimen was listed as of unknown date, but in the original publication of *C. sericea* there is the abbreviation "H. U." which I take to mean that the plant was growing in the garden at Upsala at the time the description was written. It is possible that the fruits had not matured at the time the description was written, so

the color may have been taken from the Plukenet reference, or the plant may have been a blue-fruited form. In the absence of better evidence to the contrary, the specimen in the Linnaean Herbarium must be accepted as typifying the species, giving *C. sericea* L. (1771) clear priority over *C. stolonifera* Michx. (1803).

Farwell avoided the logical consequence of this view by proving, to his satisfaction, at least, that *C. amomum* Mill. (1768) should properly apply to this species. After reading what Miller says of his *C. amomum* I cannot agree to this. Rickett, after demonstrating conclusively that *C. amomum* is correctly applied in its traditional sense, faced with the necessity of replacing *C. stolonifera* with *C. sericea*, showed that it had commonly been regarded as a synonym of *C. amomum*, as interpreted by Willdenow. This being the case, he suggested placing *C. sericea* on the list of *nomina ambigua*.

This disposition I can scarcely accept. In the first place, there is no provision in the International Rules for a method of placing things in this, as yet, non-existent list. Secondly, if this list is to be used for the disposal of any name which upsets a long established usage, it merely becomes an application of the idea of *nomina specifica conservanda* under a different name. This principle was decisively rejected by at least two congresses. If all names about which there has in the past been uncertainty or confusion are to be placed in the list of *nomina ambigua* the number of familiar names that will fall should make anyone stop and reconsider. In this case there seems scarcely even much actual confusion, since in recent years *C. sericea* has only been thought of as a synonym. The translation "silky cornel," which has been applied somewhat to *C. amomum*, is unfortunate, but is the logical result of the practice of manufacturing common names by translation of scientific names. The appellation "silky" seems to me to apply much more aptly to the appressed pubescence of the plant under discussion than to the spreading or woolly, often rusty pubescence of *C. amomum*. The consequent confusion attendant on the change of one familiar name seems to me much less than that which would follow a precedent such as placing *Cornus sericea* L. in the list of *nomina ambigua*. If no new combinations were to be made here, it would be simple, of course, to express my opinion and then continue to follow usage, leaving it to a monographer to do something about it. Since there are new combinations, I feel that it is advisable to make them under *C. sericea* L.

Unhappily, this decision was reached after I had annotated a considerable number of specimens with the appropriate epithets under *C. stolonifera*. Those combinations are to be regarded as unpublished herbarium names, which I hope no one will feel called upon to dig up and insert into the literature.

The herbarium abbreviations used with the occasional citations are those

of the last list of standardized abbreviations published in Chronica Botanica (**5**: 143–150, 1939; **6**: 377–378, 1941).

CORNUS SERICEA L., Mant. **2**: 199, 1771. *Cornus stolonifera* Michx. Fl. Bor. Am. **1**: 92, 1803.

(For detailed synonymy applying to the species, see that under the subspecies and formae.)

The distribution of this species in eastern North America lies almost entirely within the glaciated region. In the West it extends along the Rocky Mountains to Chihuahua, Mexico, through the Great Basin to the mountains of Arizona, and on the Pacific Coast from Alaska to San Diego County, California. Its northern limits have not been determined, but it is known from Newfoundland, Keewatin, Mackenzie, the Yukon Valley, and coastal Alaska.

Its closest relatives seem to be *Cornus alba* L. of northeastern Asia and *C. amomum* Mill. of the eastern United States. *C. alba* differs in having the stone longer than broad, and acute at each end. *C. amomum* differs in its brown pith and in having the stone subglobose and ribbed. *C. hessei* Koehne, of northeastern Asia, which I do not know, may be even closer to *C. sericea*.

A key to such a variable and intergrading series of forms is rather useless, as it will necessarily not work with those individuals which partake of the characters of two or more forms. However, the following will make possible the identification of flowering or fruiting material of a large majority of the plants of this species.

1. Petals 3.5–5 mm. long, stone with lateral faces sculptured, pubescence spreading except sometimes on inflorescence branches (ssp. *occidentalis*).
   2. Inflorescence with spreading hairs even on minor branches . . . f. *occidentalis*.
   2. Inflorescence with spreading hairs on peduncle only (rarely on large divisions) . . . f. *californica*.
1. Petals 2–3 mm. long, stone with lateral faces smooth, pubescence all or at least partly strigose (ssp. *stolonifera*).
   3. Under side of leaves with spreading or woolly hairs . . . f. *baileyi*.
   3. Under side of leaves strigose, except sometimes near midrib.
      4. Peduncle strigose, young twigs glabrous or thinly strigose . . . f. *stolonifera*.
      4. Peduncle with spreading hairs, young twigs tending to be woolly or densely appressed pubescent . . . f. *interior*.

Specimens are not usually cited, as they would take up much space and this discussion is not intended to be monographic. A selection of a few representative ones would perhaps give a false idea of the distribution of the forms. The synonymy cited for categories above *forma* is just that necessary to establish priority, except that the horticultural varieties have only been assigned to subspecies. This is because of uncertainty about the final disposition of these cultivated forms, and because I have not seen authentic material of most of them. For the forms all synonyms known to me which are based on wild plants are placed, some, of course, tentatively pending availability of types. Those names which have elsewhere been cited as synonyms but which are merely misapplications of names of other plants are not included.

CORNUS SERICEA ssp. **stolonifera** (Michaux) Fosberg, comb. nov. *C. alba* var. *behnschi* Schelle, Handb. Laub. Benen. 366, 1903. *C. alba* var. *coloradense* Koehne, Mitt. Deutsche Dendr. Ges. (1903): 39, 1903. *C. alba* var. *elata* Koehne, Mitt. Deutsche Dendr. Ges. (1903): 39, 1903. *C. alba* var. *elongata* Koehne, Mitt. Deutsche Dendr. Ges. (1903): 39, 1903. *C. alba* var. *nitida* Koehne, Mitt. Deutsche Dendr. Ges. (1903): 39, 1903. *C. stolonifera* var. *flaviramea* Spaeth, Mitt. Deutsche Dendr. Ges. (1903): 39, 1903. *C. alba* var. *angustipetala* Wolf, Dendr. Gart. Kais. Forst. Pet. sep. p. 2, 1907. *C. alba* var. *splendens* Demcker, Mitt. Deutsch. Dendr. Ges. (1909): 326, 327, 1909. *C. stolonifera* var. *behnschi* Schneid., Ill. Handb. Laubh. **2**: 440, 1909. *C. stolonifera* var. *elata* Schneid., Ill. Handb. Laubh. **2**: 440, 1909. *C. stolonifera* var. *elongata* Schneid., Ill. Handb. Laubh. **2**: 440, 1909. *C. stolonifera* var. *nitida* Schneid., Ill. Handb. Laubh. **2**: 440, 1909. *C. stolonifera* var. *coloradense* (Koehne) Schneid., Ill. Handb. Laubh. **2**: 440, 1909. *C. alba* ssp. *stolonifera* Wangerin, in Engl. Pflanzenr. **41**: 53, 1910. *C. stolonifera* var. *angustipetala* Schneid., Ill. Handb. Laubh. **2**: 1041, 1912. *C. stolonifera riparia* Visher, Geogr. Geol. & Biol. So. Dak. 101, 1912. *C. stolonifera* var. *splendens* Schneid., Ill. Handb. Laubh. **2**: 1041, 1912. *Svida stolonifera* var. *flaviramea* Moldenke, Cult. Pl. 25, 1938.

Pubescence various, but with some hairs appressed and attached in middle; flowers with petals 2–3 mm. long; stone with smooth lateral faces.

This subspecies is found over the whole range of the species, but is less common at lower altitudes in the far West. It may be divided into the three forms treated below.

If the varietal category is used in place of subspecies the varietal epithet *coloradense* should be chosen from among several of the same date as it is the only one based on a wild plant. The rest are cultivated. It is only with reservations that I assign to this subspecies the various horticultural plants that have been given varietal names by Koehne and his contemporaries. Since the types, if they have been preserved, are now inaccessible, it is impossible to do more than place them as well as I can from the brief diagnoses given. This is an example of the difficulties besetting the botanist after even a slight amount of horticultural selection in a species. These plants are doubtless recognizable forms which should have horticultural names.

CORNUS SERICEA f. **stolonifera** (Michx.) Fosberg, comb. nov. *C. sericea* L., Mant. **2**: 199, 1771. *C. stolonifera* Michx. Fl. Bor. Am. **1**: 92, 1803. *C. purshii* G. Don, Gen. Syst. **3**: 199, 1834. *C. alba* var. *coloradense* Koehne, Mitt. Deutsch. Dendr. Ges. (1903): 39, 1903. *C. nelsoni* Rose, Contr. U. S. Nat. Herb. **8**: 54, 1903. *Svida* (or *Suida*) *stolonifera* Rydb. Bull. Torrey Club **31**: 572, 1904. *S. stolonifera* var. *riparia* Rydb. Bull. Torrey Club **31**: 573, 1904. *Cornus alba* ssp. *stolonifera* Wangerin, Pflanzenr. **41**: 53, 1910. *C. instolonea* Nels. Bot. Gaz. **53**: 224, 1912. *C. stolonifera riparia* Visher, Geogr. Geol. & Biol. S. Dak. 101, 1912. *Ossea instolonea* Nieuwl. & Lunell, Am. Midl. Nat. **4**: 487, 1916. *Svida instolonea* Rydb., Fl. Rocky Mts. 635, 1065, 1917.

Stems red to purple, rarely greenish or even yellow, glabrate or only thinly strigose; leaves with under surface strigose, sometimes with some spreading hairs near the midrib. Inflorescence strigose, flowers with petals 2–3 mm. long; stone slightly to conspicuously oblique, usually rather com-

pressed, usually smooth on the lateral faces, variable in size. Fruit white, rarely pale blue (MAINE: Dover, Piscataquis Co. *Fernald 305* [US]).

Found from Newfoundland, Keewatin, Mackenzie (Good Hope, 66° 15′ N., *Dutilly 8993* [LCU, USNA]), and Alaska (mainly near the south coast), south to New England, New York, Pennsylvania, Ohio, Indiana, Illinois, Missouri, and in the Rocky Mountains south to New Mexico, Chihuahua (*Townsend & Barber 26* [NY], *E. W. Nelson 4927* [US; TYPE of *C. nelsoni* Rose]), Arizona, and on the Pacific Coast to Southern California. Only a few eastern specimens, all from Pennsylvania (Reading, Berks Co., *Wilkens 6186* [PENN]; Haverford, Montgomery Co., *Schaeffer 1546* [PENN]; Wynnewood Sta., Montg. Co., *Walker 1932* [PENN]; from marsh at Centre Furnace, Centre Co., *Wahl 319* [PENN]) come from outside the glaciated area in the eastern portion of the range.

In the Rocky Mountains and westward it intergrades completely in all characters with ssp. *occidentalis*, often approaching the large flowers or sculptured stone, or both, of the latter. Perhaps the few eastern specimens with a somewhat ribbed stone may be the result of hybridization with *C. amomum*. Such is one from Tioga County, Pa. (*Wood 1586* [PENN, USNA]), which also has the brown pith of *C. amomum*. In the Great Lakes region, especially in sandy places it is largely but not completely replaced by f. *baileyi* and in the western central portion of the continent, from Nebraska to the Yukon region by f. *interior*.

CORNUS SERICEA f. **baileyi** (C. & E.) Fosberg, comb. nov. *C. baileyi* Coult. & Evans, Bot. Gaz. **25**: 37, 1896. *C. alba* ssp. *baileyi* Wangerin, in Engl. Pflanzenr. **41**: 55, 1910. *C. stolonifera* var. *baileyi* Drescher, Trans. Wis. Acad. **28**: 190, 1933. *Svida baileyi* Rydb., Brittonia **1**: 94, 1931.

Twigs purple, thinly appressed hairy or somewhat woolly when young, leaves thinly woolly beneath, some hairs attached at center, veins drying brownish beneath; inflorescence with at least peduncle spreading hairy, usually becoming more or less strigose distally, stone somewhat compressed, somewhat oblique, not ridged or furrowed except sometimes on edges.

Found in reasonably typical state in Michigan, Ontario, Minnesota, and northern Indiana, usually in sandy areas around the Great Lakes. One specimen, *Rothrock* in 1868 (Ph) from Centre County, Pennsylvania, has the leaf pubescence of this form but has no spreading hairs on the peduncle.

CORNUS SERICEA f. **interior** (Rydb.) Fosberg comb. nov. *Svida interior* Rydb., Bull. Torrey Club **31**: 572, 1904. *Cornus interior* Petersen, Fl. Nebraska 163, 1912. *Ossea interior* Lunell, Am. Midl. Nat. **5**: 239, 1918. *Cornus stolonifera* var. *interior* St. John, Fl. S. E. Wash. 303, 1937.

Stems purple, noticeably close-woolly when young, leaves strigose beneath, or rarely with spreading hairs especially near midrib, veins not especially prominent beneath, drying brownish; inflorescence spreading pubescent or woolly, at least on peduncle; stone oblique, somewhat compressed, variable in size.

A middle-western form, mostly collected in Nebraska, but extending east to Lorain County, Ohio (*Ricksecker* in 1895 [US]), west to Pikes Peak Forest Reserve, Colorado (*Flintham* [US]) and Cascade County, Montana (*Palmer 36943* [US]), (*Flint 22* [USFS]), north through the Dakotas, to Wood Buffalo Park, Mackenzie Basin, Canada (*Raup 2908* [US]), and to

the Yukon Valley.[1] One specimen is available from Matanuska, coastal Alaska (*Anderson 938* [US]).

CORNUS SERICEA ssp. **occidentalis** (T. & G.) Fosberg, comb. nov. *C. sericea* var. *occidentalis* T. & G., Fl. N. Am. **1**: 652, 1840.

Pubescence largely spreading, petals about 4 mm. long, stone very oblique, furrowed or roughened on lateral faces.

Pacific coast region from Canada to Southern California. Contains the following two forms. In the synonymy for this subspecific name, only the name-bringing synonym is given. Others may be found under the respective *formae*.

CORNUS SERICEA f. **occidentalis** (T. & G.) Fosberg, comb. nov. *C. sericea* var. *occidentalis* T. & G., Fl. N. Am. **1**: 652, 1840. *C. pubescens* Nutt., Sylva **3**: 54, 1849 (not Willd. in R. & S. Syst. Mant. III: 252. 1827). *C. occidentalis* Cov., Contr. U. S. Nat. Herb. **4**: 117, 1893. *Svida pubescens* Standl., Smiths. Misc. Coll. 56 (33): 3, 1912. *Cornus californica* var. *pubescens* Macbr., Contr. Gray Herb. **56**: 54, 1918.

Branchlets green to purple, thinly spreading pubescent, leaves variable in size, spreading pubescent beneath, often densely so, inflorescence conspicuously spreading pubescent, even on smaller divisions, stone as in f. *californica* but less often ridged, sometimes scarcely compressed.

Intergrades freely with f. *californica.*

Rare in coastal California Mountains (one specimen from San Bernardino Mountains) from San Diego County north, commoner northward, as far as British Columbia, east to Idaho.

CORNUS SERICEA f. **californica** (Mey.) Fosberg, comb. nov. *C. californica* C. A. Mey., Bull. Phys.-Math. Acad. Petersb. **3**: 373, 1845. *C. torreyi* Wats., Proc. Am. Acad. **15**: 145, 1876. *C. pubescens* var. *californica* Coult. & Evans, Bot. Gaz. **15**: 37, 1890. *Svida californica* Abrams, Bull. N. Y. Bot. Gard. **6**: 429, 1910. *S. torreyi* Heller, Cat. ed. 3: 273, 1914. *Cornus californica* var. *nevadensis* Jepson, Man. Calif. Fl. Pl. 733, 1925. *C. stolonifera* var. *californica* (Mey.) McMinn, Ill. Man. Calif. Shrubs. 377, 1939.

Branchlets greenish to red or purple, leaves ample, tending to be ovate, pubescence spreading beneath, inflorescence usually not very densely pubescent, with spreading hairs at least on the peduncle, appressed on smallest divisions, flowers relatively large, stone quite variable in size and shape, usually somewhat compressed, often quite broader than long, usually very oblique, generally more or less ridged or irregular longitudinally.

Common in California except the Great Valley floor, deserts, and alpine zone; extending north to British Columbia and Idaho, east to Nevada.

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AGRICULTURE, WASHINGTON, D. C.

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[1] A series of collections made in northwestern Canada in 1940 by Dutilly may give a rough idea of the northern extension of this form. Alberta: Chipewyan, Athabaska L., 58° 45′ N., *Dutilly 8280.* British Columbia: Francois, 59° N., *Dutilly 8710.* Mackenzie: Fort Smith, Slave River, 60° N., *Dutilly 8117, 8118, 8153;* Fort Liard, 60° N., *Dutilly 8618;* Fort Simpson, Mackenzie River, 61° 50′ N., *Dutilly 8588;* Fort Norman, Mackenzie River, 64° 54′ N., *Dutilly 8838* [all LCU; some duplicates USNA].

BULLETIN OF THE TORREY BOTANICAL CLUB

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A NEW DUSSIA AND ORMOSIA AVILENSIS

JOHN H. PIERCE

The original description of *Ormosia avilensis* Pittier is bigeneric and in need of clarification. The foliage and fruit described are clearly those of *Ormosia,* while the flower is that of *Dussia.* The material collected or identified by Pittier is also bigeneric and there is further some question as to the number cited for the type specimen. The mixture of numbers, dates, and bigeneric material is so confusing that for the sake of clarity a discussion of each specimen involved is here presented.

Delgado 37, April 1937: cited by Pittier, presumably through typographical error, as the type of *O. avilensis* (in fruit); also as the type of *Pithecolobium pulchellum* Pittier (in flower). The flowering specimen so numbered is actually, in the opinion of the author, *Pithecolobium pulchellum* Pittier. No fruiting specimen of this number corresponding to the description of *O. avilensis* has been found.

Delgado 47, March 1937: identified by Pittier as *O. avilensis,* actually *Dussia coriacea* Pierce. Since Pittier cites a fruiting specimen as type of *O. avilensis* it is obvious that the original floral description was not taken from the type. This number was probably the basis for the original floral description.

Delgado 35, March 1937: both the foliage and fruit of this sheet fit the original description, if we exclude the floral description, and it is probably the actual type of *O. avilensis* Pittier.

Pittier 47, March 1937: this number consists of two sheets collected and identified by Pittier as *O. avilensis.* One sheet is *O. tovarensis* Pittier, the other consists of the foliage and fruit of *Dussia coriacea* Pierce and the seeds of *O. tovarensis* Pittier.

It seems probable that when Pittier described *O. avilensis* he had before him two or more of the specimens listed above and that he based the floral part of the description on *Delgado 47,* the rest on *Delgado 35.* This confusion can perhaps best be clarified by selecting as the LECTOTYPE of *O. avilensis Delgado 35.* The floral portion of the original description is excluded from *Ormosia* and refers to *Delgado 47,* which then becomes the TYPE of *Dussia coriacea* Pierce, described below.

O. AVILENSIS Pittier, Bol. Soc. Venez. Cien. Nat. **4**: 84. 1937. emend. Pierce (excluding the floral description). LECTOTYPE: *Delgado 35,* flowers unknown; Selvas del Avila, Venezuela.

Dussia coriacea Pierce, sp. nov. Arbor 20–25 m. alta; ramulis adultis puberulentibus; foliolis 5, oppositis, laminis lateralibus oblongo-ovatis, ter-

minalibus obovatis, 7–9 cm. longis et 3.5–6 cm. latis, basilaribus minoribus, supra glaberrimis, opacis, subtus uniforme rufo-tomentosis, venis lateralibus 10–14, prominentissimis, nervis transversalibus pluris, prominentissimis; floribus racemosis, ad apicem ramulorum novarum defoliatorum aggregatis; bracteolis ad basim calycis, obovatis acutis; calyce 5-lobo, subaequitanti; petalis lilacinis; vexillo suborbiculari, apice acutiusculo, extus plus minusve ferrugineo-pubescente; alis oblongo-lanceolatis, obliquis, minutissime pubescentibus; petalis carinalibus alis majoribus, valde inaequilateralibus, ovato-lanceolatis, infra ad suturam ferrugineo-pubescentibus; staminibus 10, ad basim leviter connatis; legumine maturo late fusiforme, fulvo-tomentoso; seminibus ignotis.

TYPE: *Delgado 47*, in flower, Selvas del Avila, Venezuela (Herb. N. Y. Bot. Gard.). The fruit characters taken from *Delgado 153* (at N. Y.).

This species is known only from the type locality and is readily distinguished from the other species of the genus by the small number of leaflets, the coriaceous texture and uniform pubescence of the leaflet and the smaller fruit. The pubescence suggests a relationship with *D. sanguinea* Kr. & Urb. while the fruit shape is close to *D. cuscatlanica* (Standl.) Standl. & Steyermark.

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NUCLEAR BEHAVIOR IN THE MUCORALES—II. THE RHIZOPUS, PHYCOMYCES AND SPORODINIA PATTERNS

VICTOR M. CUTTER, JR.[1]

In a preceding paper (8) the nuclear behavior in eight species of the Mucorales was described, and the characteristic type of behavior designated as the "*Mucor*" pattern. The results of this comparative study indicated that in these species nuclear behavior followed the same basic scheme at corresponding stages in the life cycle. The predominant characteristics of this pattern were the presence of the haploid condition throughout the mycelial stages, karyogamy and reduction division occurring before the onset of the dormant period in the zygote; and the almost immediate resumption of the haploid condition before the accumulation of reserve products takes place in the zygote. Further features of this pattern were the rather short dormant period in the zygote, 1–5 months depending upon the species concerned, and the complete segregation of sex at the time of meiosis. Thus the diploid condition in this group of species was transitory, never persisting for more than a few days. In the present paper the nuclear behavior in six additional species will be discussed. In these species, *Rhizopus nigricans* Ehrenb., *Absidia glauca* Hagem., *Tieghemella coerulea* Naumov, *Phycomyces blakesleeanus* Burgeff, *Phycomyces microsporus* Van Tieghem, and *Sporodinia grandis* Link, the nuclear behavior falls into three different categories. The same techniques previously employed (8) were used in this study, and the species were studied comparatively at all stages in the life cycle. As in the preceding paper no attempt will be made to review extensively the literature on this subject since several adequate reviews are already extant, and stages in the life cycle which have already been adequately portrayed will not be reillustrated here. Reference to these previous investigations will be made in the appropriate places.

RHIZOPUS NIGRICANS Ehrenberg. This is undoubtedly the most frequently observed and familiar species in this order. It was with *Rhizopus nigricans* that Blakeslee initiated his brilliant investigations which led to the concept of heterothallism and homothallism in the lower fungi, and to the realization that the formation of zygospores in the Mucorales was fundamentally a sexual process. The early work on this problem has been admirably summarized by Blakeslee (2).

[1] Publication of the figures in this and the previous paper by Dr. Cutter was made possible by the Lucien M. Underwood Memorial Fund. Pages in excess of the usual number were printed at the expense of the author.

The details of nuclear behavior and sporangium formation in the asexual phase of this fungus were very accurately described by Swingle (25) and his description confirmed by Moreau (20). The results of the present study as far as the asexual stages are concerned, are in complete agreement with those of Swingle and Moreau, and no further attention will be devoted to them here.

Cytological investigations on the nuclear behavior in the sexual phase have been carried out by Namyslowski (21), Macormick (19), Moreau (20), and Baird (1). Namyslowski reports the presence of numerous nuclei in the young zygospore, but has not succeeded in distinguishing any nuclear fusion. Macormick, in a preliminary note on her research, reports that numerous nuclei enter the young coenozygote, and there increase in size. Thereupon, all the nuclei except two disintegrate, and these remain embedded in a coenocentrum. This coenocentrum persists until quite late in the development of the zygospore but in mature zygospores there are many nuclei of the same size as those in the mycelium. She is unable to interpret this condition nor does she indicate her conception of a mature zygospore. Moreau does not confirm Macormick's observations and feels that the nuclear behavior in *Rhizopus* differs in no fundamental way from the other members of the Mucoraceae which he has studied. He figures multiple nuclear fusions accompanied by the degeneration of unfused nuclei. He does not follow the ultimate fate of the fusion nuclei, but it is clear that he believes the meiotic process to occur at the time of zygospore germination.

Baird concerns himself more with the behavior of the individual nucleus in *Rhizopus* and the changes which it undergoes during the life cycle than with an investigation of the changes and relationships of the entire nuclear complement. He states that the nuclear behavior in *Rhizopus* is essentially the same as that described by him in *Phycomyces*. In neither of these forms has he been able to discern nuclear fusions at any stage. None of these authors has studied the nuclear condition in the germinating zygospores. This germination process has been reported only by Blakeslee (3) who observed it twice.

Callen (7), working with a recently described homothallic species, has traced the nuclear behavior through the development of the zygospore but he was unable to obtain germination of these spores. In this species, *Rhizopus sexualis* (Smith) Callen, the nuclei associate in pairs during the formation of the coenozygote but he feels that it is doubtful whether any nuclear fusion occurs. Nuclear degeneration also takes place at this stage but ceases as the zygote matures. The nuclei remain in the paired condition as the zygote becomes dormant. Callen's account of nuclear behavior in *R. sexualis* is quite reminiscent of the nuclear cycle described below for two species of *Phycomyces,* and perhaps will prove to be an intermediate form between the *Rhizopus* and *Phycomyces* patterns.

The most striking difference between the nuclear condition in the progametangia and young coenozygote of *Rhizopus* and that seen in the species studied previously is the vast number of nuclei present per given unit of cytoplasm. These nuclei are rather large and appear proportionately far more numerous than in the corresponding stages of other species. Mitotic divisions in this species present the same general aspect as those described elsewhere (8). Centrosomes are rather regularly present, and the achromatic spindle is fairly well developed. No discrete chromosomes have been distinguished in any of the division stages yet encountered.

The young progametangia develop slowly and the suspensors are usually not delimited until the second day after contact has been established between the zygosporic hyphae (fig. 4). The wall separating the suspensors from the gametangia is formed by a cleavage furrow which arises at the periphery of the progametangial cell and progresses towards the interior (fig. 5). Both Moreau and Macormick have stated that a difference in the staining reaction of the two gametangia can be demonstrated, even after the formation of the coenozygote. If this is true, it is a point of great significance, since it would constitute good evidence of a physiological differentiation between the plasmas of the two opposed strains. These observations are not substantiated in the present study, and any differential staining reaction of the cytoplasm of the gametangia appears purely coincidental.

Development of the coenozygote is slower than in the species previously observed. During this period mitotic nuclear divisions occur with great rapidity and tremendous numbers of unfused, expanded nuclei are present in the coenozygote as the exospore is formed. At this stage karyogamy occurs between many of the nuclei present in the cell, but a large number of the supernumerary nuclei do not engage in fusion and remain for some time in the unfused condition. Karyogamy differs somewhat from that described in *Mucor genevensis* and *Blakeslea trispora* (8) in that the paired nuclei do not enlarge greatly before fusion, nor do these nuclei remain associated in pairs for any length of time before fusion occurs. The fusion or diploid nuclei can be distinguished readily from the unfused nuclei by their greater size and more prominent reticulum and central body (fig. 7). In the suspensors the nuclei are very prominent at this stage, but shortly assume the unexpanded phase, and ultimately degenerate.

Both fused and unfused nuclei remain in evidence for several days until the endospore has been deposited. As this endospore is developed the unfused nuclei begin to degenerate, and before the dormant condition is reached all the unfused nuclei have apparently disintegrated. This degeneration is initiated in some cases at least by well marked amitotic divisions which are frequent during this period. Zygospores ten days old have become, to all appearances, dormant.

The oil plastids are not particularly prominent in *Rhizopus,* but can occasionally be demonstrated. On the other hand, mucorine crystals are extremely abundant and are quite frequently aggregated into groups. These groups of crystalline structures may be synonymous with the structures which Macormick identifies as coenocentra. Aside from this no evidence of coenocentra have been observed. There is a considerable amount of oil present in the vacuoles which arises in the cytoplasm as nuclear degeneration commences. It seems likely that the degeneration of these unfused nuclei may augment this supply of oil, if not accounting for all of it.

During the dormant period the fused nuclei assume the unexpanded state but are still conspicuously larger than the nuclei present in mycelia and gametangia (fig. 8). The oil reserve of the dormant zygospores is largely confined to the central vacuoles which have arisen by the confluence of many smaller ones. The cytoplasm becomes restricted to a thin peripheral layer and the mucorine crystals largely disappear. The exospore and endospore of the dormant zygotes is extremely thick; *Rhizopus* is by far the heaviest-walled species yet observed in the Mucorales. The dormant period persists for approximately a year, during which no visible change occurs in the spore contents.

The difficulty of germinating the zygospores of *Rhizopus* has become almost proverbial among mycologists who have worked with this species. Blakeslee succeeded in inducing zygospores to germinate only twice, in both cases after a rest period of more than a year. These germinations, however, did not result in the production of normal sporangiophores and spores. During the course of this study a number of zygospores of this species have been germinated, but germinations have never proceeded normally. Whenever germinations were obtained only abortive germ tubes were produced, and neither sporangia nor mycelia were developed by these tubes. Further remarks upon this subject will be presented in a subsequent publication.

The nuclear condition observed during germination differs markedly from that described in *Mucor genevensis* (8). At the onset of renewed activity in the cytoplasm, 2–3 days before germination, the cytoplasm, which has been restricted to a thin layer adhering to the endospore wall, begins to increase in extent and becomes vacuolate, with the oil reserve, previously restricted to the central vacuoles, now becoming dispersed throughout the cell. This oil interferes with precise staining of the nuclei at this period but nuclear division apparently takes place rapidly, for as the oil disappears many small expanded nuclei can be discerned, arranged in the peripheral regions of the spore. There are still a few fusion nuclei present at this stage but they all disappear before the formation of the germ tube. The smaller nuclei are several times more numerous than were the fusion nuclei during the dormant period. These unfused nuclei undergo mitotic divisions as the

germ tube develops. The heavy zygospore walls of *Rhizopus* do not rupture as widely as in other species where this process has been observed, and the germ tube gains access to the exterior through a rather restricted pore (fig. 9). This germ tube grows slowly and a few mitotic divisions can be seen in the developing tube. At the end of several days' growth, at which time the tube may have reached a length of 1 centimeter, it slowly shrivels and cytoplasmic activity ceases, even when the tube is in contact with nutrient media. It appears from the results outlined above, that the reduction process must occur in the first divisions of the fusion nuclei as renewed growth of the cytoplasm takes place in the germinating spore, since the nuclei, after these first few divisions, are notably smaller than those present in the dormant zygospore. However, no other indications have, as yet, been seen which were suggestive of meiotic divisions, possibly because of the difficulties in distinguishing nuclei in the oil soaked cytoplasm of the early germination stages. If this interpretation is correct, *Rhizopus* shows a somewhat advanced condition over those species described under the "*Mucor*" pattern, where the diploid condition is present for a few days at most, and where reduction division presumably occurs before the dormant condition is reached. In *Rhizopus* the diploid condition seems to be prolonged until the onset of germination.

ABSIDIA GLAUCA Hagem. This heterothallic species has been investigated cytologically only by Ling Young (18) although Moreau (20) has studied *Absidia orchidis* which is very closely related to and perhaps synonymous with it. Both these authors agree that in the younger stages the nuclei are very minute and appear even more numerous than is usually the case in the Mucorales. Moreau describes prominent fusion nuclei which appear after the formation of the coenozygote, and he states that many supernumerary nuclei disintegrate. Ling Young corroborates Moreau in this. The older stages of the zygospore and the germination stages have not yet been described, as far as the author is aware. Moreau investigated the asexual reproduction stages and noted that the sporangiospores were uninucleate. He describes the process of spore formation as similar to that described by him for *Mucor spinescens* (20).

As far as could be determined in this study, the development of the sporangiophore and sporangium, and sporangiospore formation differed in no way from that previously described for *Absidia spinosa* (8). The nuclei are somewhat smaller and proportionately more numerous than in the latter species. The progametangia develop in the usual way and are supplied with very numerous expanded nuclei. The suspensor appendages develop as in *A. spinosa*, but in this species are produced in about equal numbers on both suspensors, and are not confined to the larger suspensor as previously re-

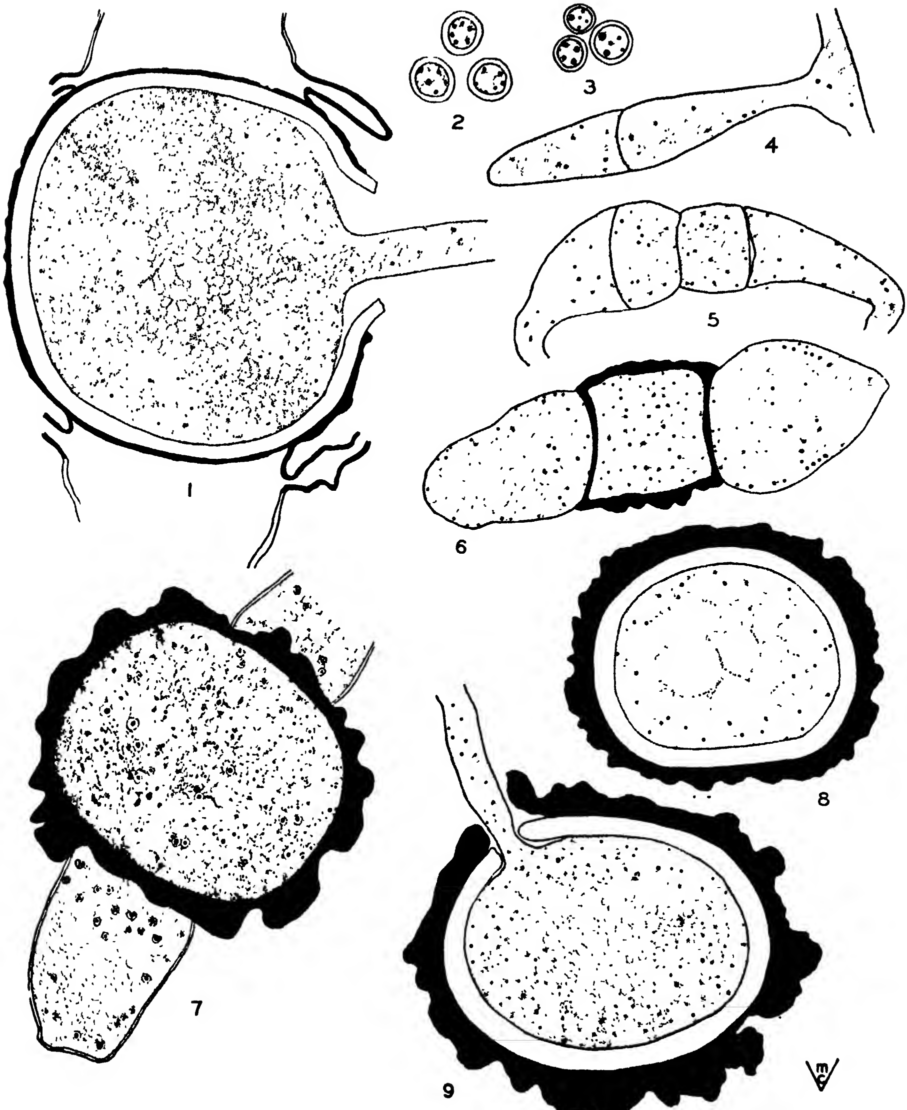

FIG. 1. *P. blakesleeanus*, germinating zygospore (Flemming's; Haematoxylin). ×170. FIG. 2. *P. blakesleeanus*. Sporangiospores from asexual sporangium; unfused unexpanded nuclei (F.A.A.; Buffer). ×960. FIG. 3. Sporangiospores from germ sporangium. Fused and unfused unexpanded nuclei (F.A.A.; Buffer). ×960. FIG. 4. *Rhizopus nigricans*, progametangia 7 hours old (Flemming's; Haematoxylin). ×400. FIG. 5. Gametangia 24 hours old (Flemming's; Haematoxylin). ×400. FIG. 6. Zygospore 36 hours old, unfused expanded nuclei (Craf; Cahal-Brozek). ×400. FIG. 7. Zygospore 3 days old, fused and unfused nuclei. Note prominent unfused nuclei in suspensors (Flemming's; Triple). ×400. FIG. 8. Dormant zygospore, fused unexpanded nuclei (Gilson's; Feulgen). ×400. FIG. 9. Germinating zygospore 13 months old, fused and unfused nuclei expanded (Flemming's; Triple). ×400.

ported in *A. spinosa*. In *A. orchidis* Lendner (17) has illustrated copulation between the young appendages produced from the two suspensors of a zygospore, thus forming a secondary zygospore upon appendages which here functioned as zygosporic or sexual mycelium. The same situation has been observed several times in our cultures of *A. glauca* and indicates clearly that there is a localization of oppositely sexed plasma in the suspensors as well as in the gametangia.

As in *Rhizopus nigricans*, nuclear divisions continue with great rapidity in the developing coenozygote and karyogamies are not in evidence until the exospore has been formed. The fusion nuclei are large and prominent, but they are of the *Rhizopus* type rather than of the type encountered in the species showing the "*Mucor*" pattern. All the nuclei present in the young zygospore do not fuse, and for several days after karyogamy has occurred, the degeneration of unfused nuclei may be observed. Apparently all supernumerary nuclei degenerate before the zygospore becomes dormant.

In the dormant zygospore, because of the great abundance of oil plastids. it is difficult to ascertain whether all the nuclei remain in the fused condition, but no evidence of nuclear division of any sort has been seen between the time when the zygospore becomes dormant and the onset of germination. Presumably the nuclear condition during the dormant period is similar to that in *Rhizopus* where the diploid unexpanded phase persists until the spore germinates. Germination in our material occurred between the sixth and seventh month after the formation of the zygospore. The nuclear condition in the germinating spores is identical with that described in *Rhizopus*, and apparently meiosis takes place during the first few divisions of the nuclei before the germ tube is formed. The germ tube develops into a long stolon which bears a fasicle of sporangia in the same manner as the vegetative mycelium. The sex of all the spores in any germ sporangium is either plus or minus. Segregation of sex is thus apparently complete at meiosis.

The similarity of nuclear behavior in *Absidia glauca* with that in corresponding stages of *Rhizopus nigricans* leads to the conclusion that the relationships of the genus *Tieghemella*, erected by Berlese and de Toni to accommodate the species previously included in *Absidia* which resemble *A. orchidis* and *A. glauca*, are closer to *Rhizopus* than they are to the *Hagemia* section of *Mucor* and the *Absidia spinosa* type. In this material it is not possible to corroborate Moreau's conception (20) that *A. glauca* shows essentially the same cytological behavior as *A. spinosa*.

TIEGHEMELLA COERULEA (Bainier) Naumov. This is another heterothallic species segregated from *Absidia* by Naumov. It is distinguished from *Absidia glauca* only by minor differences in mycelium color and spore size. It has not been previously investigated cytologically. In the strain used in

the present study the young mycelia of the plus strain is pure white in color and the young mycelia of the minus strain cerulean blue. This constant color difference between the sexes renders this material particularly favorable for studies concerning sexual segregation, and investigations are being carried out along this line.

From a study of a large number of zygospores it appears that in this species the appendages are developed on both suspensors less frequently than in *A. glauca*. In this respect the species may be intermediate between *A. spinosa* and the forms in the genus *Tieghemella*. As far as can be determined the nuclear behavior differs in no way from that already described in *A. glauca* and, therefore, is obviously different from that in *A. spinosa*.

PHYCOMYCES BLAKESLEEANUS Burgeff. The genus *Phycomyces* has been very thoroughly investigated by many workers. Blakeslee (2) first demonstrated heterothallism in *P. nitens* and tested the sexual condition of the sporangiospores produced in the germ sporangia. His work has been confirmed by Burgeff (4) and others. Burgeff (6) has discussed the taxonomy of the species used in the present investigation and indicates that the laboratory strain of this fungus which had been widely distributed as *Phycomyces nitens* Kunze represents in reality an undescribed species named by him *P. blakesleeanus*. *P. nitens* Kunze is a rare and obscure species, and has probably never been investigated cytologically. All these workers agree that in the germ sporangium of *P. blakesleeanus* segregation of sex is not complete, and sporangiospores of plus, minus, and homothallic or bisexual potentialities are produced. Burgeff (5) in a very extensive study of this species and its various mutants, has advanced the hypothesis that the heterocaryotic nature of some of these sporangiospores is due to a partial diploid condition, resulting from only a partial reduction of the fusion nuclei in the zygospore. Krafcyzk (15) in observations upon a similar phenomenon in *Pilobolus crystallinus*, has substantiated this theory. Burgeff (5) has also presented a brief description of the nuclear behavior throughout the life cycle. He finds that in all the early zygote stages many unfused nuclei are present; these persist in an apparently unfused condition throughout the development of the zygospore and during the dormant period. He has not been able to demonstrate a nuclear membrane on these nuclei and describes them as consisting merely of a single homogeneous chromatin body without any "nucleolus." No fusions are noted until the time of germination, when the nuclei in the peripheral regions of the spore arrange themselves in pairs and fuse. These fusion nuclei are characterized by the presence of a nuclear membrane and a "nucleolus" and are larger than the unfused or haploid nuclei. He states that the diploid nuclei always have a membrane and

nucleolus, and that he uses this character to distinguish them from the haploid nuclei.

As the germ tube develops and forms the germ sporangium fundament "mitotic" divisions occur. Burgeff states that this mitotic process is very difficult to follow. He has observed prophases in which the nucleolus disappears, and later stages in which there are 24 chromosomes which have been derived from the single chromatin body of the nucleus. He finds that the division figures are intranuclear, and that 12 chromosomes move to each pole, where they assume a new membrane. He did not observe metaphases. These mitoses are interpreted as the heterotypic division, and are followed by a homeotypic division in which distinct chromosomes are again visible. Successive divisions then occur which give rise to a large number of nuclei which lack membranes. There are now present in the sporangial fundament nuclei with and without membranes, as well as presumably unreduced diploid nuclei. Theoretically spores which receive these unreduced nuclei give rise to heterocaryotic mycelium. Spore formation in the germ sporangium, according to Burgeff's description, differs from the condition reported by Swingle (25) in the vegetative sporangia. Burgeff finds only a single nucleus entering each spore initial which then becomes multinucleate by continued nuclear divisions, whereas Swingle reports that the spore initials are multinucleate from the time they are delimited by vacuoles, and he has been unable to observe any nuclear divisions within the spore initials. Burgeff has not illustrated any of the nuclear stages, and the only stain which he has employed is iron alum–haematoxylin, which is notoriously inefficient when much reserve material is present.

Moreau (20) has carried out cytological studies on the formation of the sporangia in *Phycomyces nitens,* where his results are in accord with those of Swingle (25), and upon the early stages of the zygospore where he reports nuclear fusions occurring at the stage where the exospore is deposited. These fusion nuclei are small and hard to distinguish from the unfused nuclei. Their ultimate behavior has not been determined. Keene (14) has investigated the nuclear condition in the zygospores of the same species up to 6 months of age but has not observed germination. She reports that in the young coenozygote the nuclei associate into groups of 12–16, after which a fusion of the nuclei in pairs results in a decrease in the number of nuclei and a slight increase in the size of the fusion nucleus. She finds that in zygospores six months old nuclei of the "typical" form are present in much reduced numbers in the thin parietal layer of cytoplasm in the dormant zygospore. Her illustrations of the early stages are very accurate, and for that reason these stages will not be reillustrated here.

Baird (1) has studied the nature of the nucleus of *P. nitens* and the changes it undergoes throughout the life cycle. He finds no nuclear fusions

at any stage and can discern no evidence of nuclear divisions in the zygospore or germ tubes. He notes, however, the grouping of the nuclei in the formative stages of the coenozygote, previously mentioned by Keene. He also describes the process of vacuole formation, and indicates that the vacuoles arise within the nuclei; furthermore, he believes these vacuoles function in nuclear division. His account is confusing and seems based upon insufficient evidence. Ling Young (18) has also noted the presence of grouped nuclei in the early zygospore stages, and he reports fusions occurring after this stage. The unfused nuclei then degenerate, and function in the nourishment of the fusion nuclei. He does not investigate the further development of the zygospore.

Most accounts of the development of the progametangia and young coenozygote of this species are in agreement. These organs are multinucleate, mitotic divisions are in evidence as they increase in size, and the nuclei are extremely numerous. The development of the suspensor appendages is the same as that already described in *Absidia,* save that in *Phycomyces* they are branched. The individual nucleus in *Phycomyces* is very small when compared with such species as *Mucor genevensis* and *Blakeslea trispora,* rarely measuring more than two microns in diameter, in the unfused condition. Only Burgeff has been unable to demonstrate a nuclear membrane on the unfused nuclei at these early stages. His use of Juel's fixative may account for the discrepancy since this reagent, which contains a high percentage of alcohol, has proved quite unsatisfactory for the precise fixation of these small nuclei. As the last remnants of the wall which separates the gametangia are absorbed, the nuclei in the coenozygote come together into very characteristic groups. The nuclei are still expanded and these large nuclear groups might, in improperly fixed material, be mistaken for fusion nuclei. As far as can be determined, no karyogamy takes place at this or the immediately succeeding stages. At this time mucorine crystals become very abundant in the cell and even in the suspensors. Shortly afterwards, oil plastids are distinguished, although these do not become as prominent as in *Absidia spinosa* and *Mucor genevensis.* Keene, Moreau, and Ling Young have all reported nuclear fusions occurring soon after the nuclei associate in groups, but they all indicate that this fusion does not involve all the nuclei in the cell and that the unfused nuclei degenerate. In the present material no indication of either nuclear divisions, fusions, or degenerations can be distinguished after the nuclei have become aggregated into groups. To all appearances the nuclear condition in the zygospore remains unchanged until the onset of dormancy. As the zygospores enter the resting period after the endospore has been deposited, the only change visible in the nuclear condition is the apparent loss of the nuclear membranes. This is usual in all species at this stage when the nuclei change from the expanded to the unexpanded stage

coincident with a decrease in cytoplasmic activity (8). The grouped arrangement of the nuclei seems to be retained throughout the dormant period, which in this species persists for approximately six months. Blakeslee (2), Orban (22), and Burgeff (4) have discussed the duration of the dormant period, and the time during which the zygospores remain viable. From their observations and the results of the present investigation, it seems safe to conclude that the zygospores are capable of germinating at any time between the sixth and thirteenth month after their formation. Zygospores ready to germinate may be recognized externally by the mottled or translucent appearance of the exospore wall.

In the period just preceding the germination of the zygospores very clearly defined nuclear fusions occur. These do not, however, involve all the nuclei present in the cell. Estimating the proportion of nuclei which undergo karyogamy at this stage is a somewhat doubtful procedure, but possibly half the nuclei become enlarged to the extent where it seems reasonable to assume that they represent diploid nuclei. The fusion is of the *Rhizopus* type and the nuclei do not enlarge greatly before fusion. The grouped arrangement of the nuclei noticeable during the preceding stages of zygospore development is now lost and the fused and unfused nuclei are scattered without regularity in the peripheral regions of the cell. The central region previously occupied by several large vacuoles becomes alveolate, but remains sterile as far as the nuclei are concerned. One or two days after fusion has occurred the zygospore walls are ruptured and a thick germ tube is pushed out (fig. 1). During the early stages of tube formation mitotic divisions of both the fused and unfused nuclei are in evidence, and both types of nuclei are present in the growing tube and in the fundament of the germ sporangium. It appears probable that the meiotic divisions in at least a portion of the diploid nuclei occur at this stage as Burgeff has indicated, but as yet characteristic division stages which might be interpreted as meiosis have not been observed in this material. All observations made upon spore formation in the germ sporangia indicate that these structures are formed in exactly the same manner which Swingle (25) has described for the asexual sporangia. The spore initials are, except in an occasional instance, multinucleate from their inception, and Burgeff's statement that they are at first uninucleate cannot be substantiated. Nuclei of two sizes are certainly present in a portion of the spores of the germ sporangium (fig. 3), but there is no regularity whatever in this feature. It is presumed that both fused and unfused nuclei are distributed by chance in the spore initials, the ultimate distribution of the two types of nuclei probably forming the basis for the sexual character of the ensuing spores and mycelium. No indications have been found of nuclear division of any type within the spore initials or the maturing spores. If part of the diploid nuclei present in the germinating zygospore have undergone

meiosis in the germ tube or the fundament of the germ sporangium, then it becomes apparent that nuclei of three types must be incorporated in the spores of this sporangium; unfused nuclei which have persisted throughout the development of the zygospore, unreduced fusion nuclei, and nuclei which have undergone reduction division. The sexual character of the mycelium resulting from the germination of these spores is then determined by the balance existing between the potentially different nuclei present in them.

Spores which contain a predominance of unreduced or diploid nuclei, or a perfect balance of plus and minus nuclei presumably produce upon germination a potentially homothallic mycelium in which further sexual segregation occurs at a later date. Mycelium arising from spores which contain a majority of unreduced or haploid nuclei is influenced in its sexual behavior by the balance existing between nuclei of the opposite sexes. This condition will, of course, give rise to mycelia in which all intergradations of sexual potency, from strongly unisexual to strongly bisexual, can occur. The work of Blakeslee (2), Burgeff (5), and Krafcyzk (15) indicates that such sexual intergradation does occur in Mucors which show this incomplete segregation. It must be emphasized, however, that to date the only available cytological evidence that confirms such an hypothesis, is Burgeff's report of partial reduction of fused nuclei in the germ tube and young germ sporangium, and the present results which indicate that nuclei of two types, fused and unfused, are present in the spores of the germ sporangium. Further investigation of these critical stages is necessary in order to completely clarify the nature of the meiotic process.

In the hope of tracing the ultimate behavior of the unreduced nuclei which enter some of the spores of the germ sporangium, a great number of single-spore cultures of these spores were made. Some of these resulted in homothallic mycelia characterized by the production of pseudophores and homothallic zygospores as described by Blakeslee (2) and Burgeff (4). Homothallism of this type is, of course, phenotypic, as contrasted with the genotypic homothallism present in such genera as *Zygorhynchus* and *Sporodinia*. Development of the homothallic mycelia and zygospores of the F_1 generation as far as nuclear behavior was concerned apparently followed the same course described for the heterothallic cultures. Segregation of sex in the germ sporangium was again only partial, plus, minus, and neutral or homothallic mycelia again being obtained in the F_2 generation. The homothallic mycelia of this third generation was so weak in these cultures that no perfect zygospores were developed. It was therefore impossible to continue the study of homothallic segregation beyond the third generation. It would be expected from the fact that two types of nuclei were present in the germ spores of the first generation that these two types of nuclei would be in evidence in some of the early stages of the F_1 homothallic mycelia, but

in spite of particular efforts, no evidence of a heterocaryotic condition could be found in either the second or third generation except at the fusion stages in the zygospore of the germ tube and young germ sporangium and in the spores resulting from this germ sporangium. It is possible that the diploid nuclei present in these spores are reduced in the first few divisions after the spores germinate, and that second generation homothallism results from a perfect balance between plus and minus nuclei, rather than from a retention of the diploid condition, in some isolates. It is, of course, also conceivable that these diploid nuclei may degenerate in the early mycelial stages. As yet no technique for the adequate examination of these early mycelial stages of the homothallic segregants has been developed, since it is as yet impossible to determine the sexual character of any isolate until the reproductive organs are formed.

Observations on the asexual sporangia of heterothallic and homothallic isolates indicate that development proceeds in complete accordance with Swingle's (25) description; hence no further attention will be given to this phase of the life cycle.

PHYCOMYCES MICROSPORUS Van Tieghem. Material of this species was collected in North Carolina by G. A. Christenberry, who kindly contributed cultures for study. The fungus is smaller in all its dimensions than *P. blakesleeanus* but differs from the latter in no other important respects. It has not been previously investigated cytologically. No differences were observed between the nuclear condition in this species and in *P. blakesleeanus*, and the behavior of the spores in the germ sporangia were identical in their sexual expression. The zygospores germinate in 3–4 months, which indicates a somewhat shorter dormant period than in the aforementioned species.

SPORODINIA GRANDIS Link. Dangeard and Leger (10) were the first to investigate the nuclear condition in *Sporodinia*. They describe the young zygospore as containing many nuclei in a dense protoplasm; at a somewhat later stage nuclei of two sizes appear, and nuclei remain evident in the older stages of the zygospore as oil begins to accumulate. Leger continued this work independently (16) and described a number of anomalous bodies not observed by other workers. According to Harper (12), Lendner (17), and Ramsbottom (23), Leger's results must be questioned since they were obtained only by gross dissection with needles, and in crushed mounts, and these methods are obviously unsatisfactory for such minute structures. Dangeard (9), after a study of the nuclear condition in *Mucor fragilis*, returned to *Sporodinia*, and in light of the nuclear condition in *Mucor* he claimed a similar condition existed in *Sporodinia*, with the exception that the nuclei were very much more numerous. According to this interpretation,

nuclear fusions occurred in the maturing zygospore, and there were present in the older spores certain masses of mucorine crystals which resembled coenocentra. Moreau (20) has corroborated, in its essentials, the work of Dangeard.

Gruber (11) found numerous nuclei present in the early stages of the zygospore, and stated that there was a brief period of nuclear zonation in the young zygote, similar to that noted in many Peronosporaceae, the nuclei being more numerous in the parietal layer during this period. He found no disorganization of nuclei and no nuclear fusion, although he assumed that the latter took place at some stage in the life cycle.

Lendner (17) investigated the formation of the zygospores up to the point where the endospore is laid down. His account of the early stages agrees with those of other workers. However, he finds two large nuclei present in the newly formed zygospore, in addition to a great number of small nuclei lying scattered throughout the cell. These small nuclei divide and seem to be more numerous near the walls of the spore where they may function in the formation of the zygospore membrane. The two large nuclei fuse and occupy the exact center of the spore. He does not determine the fate of this fusion nucleus. Ramsbottom (23) has presented a very complete summary of the work on *Sporodinia* up to this point.

In 1914 Keene (13) published the results of an extensive study of the nuclear behavior in this species. She reports progressive nuclear fusions occurring as the protoplasm of the gametangia mingles, and as a result two types of nuclei are present in the young zygospore. The larger of these represent fusion nuclei. The unfused nuclei ultimately degenerate. She examined zygospores up to one year of age and in the older stages describes the formation of oil plastids which she likens to the elaioplasts of higher plants. In the mature zygospores, from 3 to 12 months old, there are numerous fusion nuclei present in the thin parietal cytoplasm which surrounds the large central vacuole. She states that this condition persists until germination, although she did not obtain germination. Mucorine crystals are reported in all the earlier stages.

Ling Young (18) has added a few points of interest to the accounts of other investigators on *Sporodinia*. He notes a tendency for the nuclei of the progametangia to aggregate into groups near the membrane which separates the gametangia. Fusions presumably take place during the formation of the young zygospore, but he states that it is difficult to distinguish the fusion nuclei, which are only slightly larger and more dense than the ordinary vegetative nuclei. He does not trace the ultimate behavior of the nuclei in the maturing spore. In azygospores of *Sporodinia*, where no fusion of protoplasts has taken place, there are numerous mitotic divisions as the spore develops and the nuclei aggregate into groups. It is difficult to ascertain

whether they fuse, but in the older spores there is a decreased number of nuclei and some difference in nuclear size. He does not feel that there is any fundamental difference between the nuclear behavior in zygospores and azygospores. Harper (12) has described the process of sporangiospore formation in the asexual sporangia of this genus. He finds that the protoplasm of the sporangial fundament segments into multinucleate portions which at maturity assume a wall and function as spores.

The early stages in the development of the thallus and azygospores have been very accurately worked out by Keene (13) and no further discussion of them is necessary. During nuclear division an achromatic spindle is developed, but it is very faint and centrioles can only occasionally be distinguished at the poles (fig. 19). At the tips of developing hyphae, sporangiophores, and zygophores threadlike mitochondria can be demonstrated by the use of basic fixatives (fig. 16). These appear closely similar to those already described in ***Mucor genevensis*** (8).

Mitotic nuclear divisions occur repeatedly during the development of the progametangia. As they reach their maximum size a darker staining area appears in the cytoplasm around the point of contact of these progametangia, extending some distance back into them (fig. 10). This presumably results from the diffusion of some enzymatic substance which causes the dissolution of the membrane separating the progametangia. It disappears shortly after the membrane has broken down (fig. 12). At this point Keene has described nuclear fusions which result in the presence of nuclei of two sizes, although she points out that the minute size of the nuclei make it difficult to determine whether there are true fusions or only divisions. The results of the present study show no evidence of any fusions occurring at this stage. No significant difference in nuclear size was seen which could not be accounted for on the assumption that certain of the nuclei have commenced to degenerate and hence are in the unexpanded phase. Mucorine crystals are sometimes very abundant and in other cases almost lacking. These crystals may at times be confused with the achromatic spindles of dividing nuclei in material stained in haematoxylin, but show no reaction to the Feulgen test and can thus be distinguished easily. Although many hundreds of spores have been examined no indication of the large fusion nucleus reported by Lendner has been seen. Figure 12 shows a young coenozygote just after the membrane which separated the progametangia has been absorbed. At the center of the spore the dark portion, which actually represents the remnants of this membrane with several included mucorine crystals, might, in material stained with Safranin such as Lendner used, he interpreted as a fusion nucleus. This figure corresponds well with his illustration of this fusion nucleus, and perhaps explains his interpretation.

Shortly after the formation of the young zygospore, the cytoplasm be-

comes denser, and through repeated mitotic divisions, the nuclei become much more abundant. At the same time the exospore is laid down. Keene has attributed the darkening of the cytoplasm to the presence of disorganizing nuclei, but in my preparations the nuclei do not appear to degenerate until a much later stage. In material fixed with basic fluids and stained in haematoxylin mitochondria are very numerous and group around the boundaries of the developing vacuoles, where they form a very characteristic reticulum, in the interstices of which the nuclei are suspended. Shortly thereafter oil accumulates in these vacuoles and the mitochondria begin to disappear. This would suggest that these structures function in the secretion of oil, a fact borne out by the researches of Tarwidowa (26) who described fat droplets as arising from the chondriome in *Basidiobolus ranarum.* Several authors have described a zonation of the nuclei in the zygospore at this time, and Keene suggests that the unfused nuclei gather near the periphery of the spore and there disintegrate. In material which had been thoroughly penetrated by the fixing fluids, no zonation of nuclei was ever encountered in this study, but when fixation had been too rapid and good penetration had not been secured, the nuclei frequently had a zoned aspect in the zygospore. Inasmuch as the nuclei near the periphery had in these cases been adequately preserved, their structure was apparent and they could be seen to be unfused, whereas those nuclei near the center of the spore were very poorly preserved and their size could not be definitely determined. Thus a fixation "artifact" may have been responsible for the description of a zonation stage in the zygospore.

About five days after the formation of the exospore, the heavy hyaline endospore begins to appear and in zygospores one week old the endospore is almost invariably present. By this time the reserve substances have become abundant and are accumulated in one or more large vacuoles in the center of the spore, while the cytoplasm is restricted to a thick parietal layer. As the cytoplasm becomes contracted and its activity ceases, the nuclei change to the unexpanded state. It seems probable that here a certain amount of nuclear degeneration occurs, since in the restricted cytoplasm the nuclei appear no more numerous than they do in the younger stages (fig. 14). A few amitotic divisions have been observed at this stage which tends to bear out the contention (8) that amitosis may be the first indication of nuclear degeneration. The large oil plastids have arisen by the coalescence of many smaller ones in the manner described by Keene (13). No further changes take place in the spore until germination ensues.

Blakeslee (2) has reported the germination of the zygospores of this species and established the fact that the sporangia arising from these germinations bear spores which are all homothallic. His results indicate that germination usually occurs within two months after the formation of the

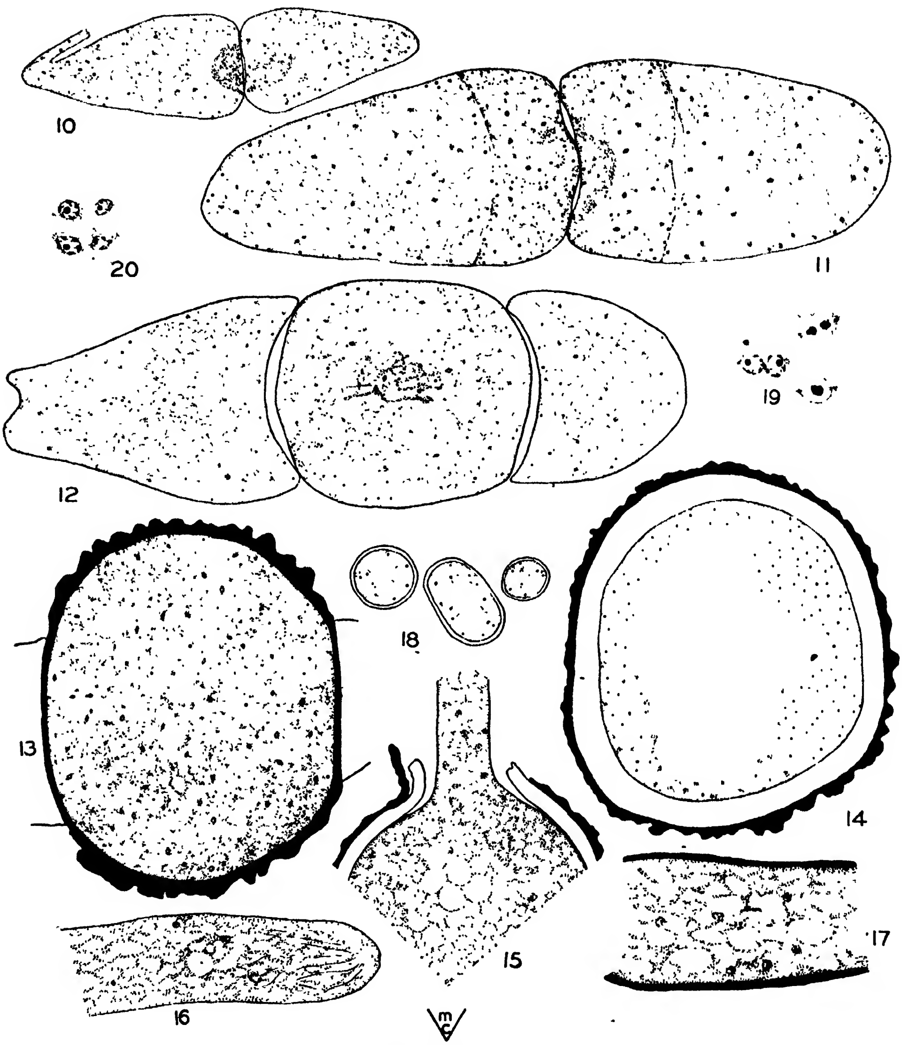

FIG. 10. *Sporodinia grandis.* Progametangia 4 hours old (F.A.A.; Haematoxylin). ×170. FIG. 11. Progametangia 8 hours old, the angular bodies are mucorine crystals (F.A.A.; Cahal-Brozek). ×170. FIG. 12. Coenozygote 10 hours old, the dark material in center of spore represents remnants of progametangial membrane (F.A.A.; Buffer). ×170. FIG. 13. Zygospore 5 days old, mucorine crystals and oil vacuoles present (Flemming's; Haematoxylin). ×170. FIG. 14. Dormant zygospore 12 days old (S.M.C.; Feulgen). ×170. FIG. 15. Portion of germinating zygospore and germ tube 17 days old (Flemming's; Haematoxylin). ×170. FIG. 16. Tip of vegetative hyphae showing threadlike mitochondria and expanded nuclei (Z.R.C.; Haematoxylin). ×660. FIG. 17. Portion of zygospore showing heavy wall and expanded nuclei (Flemming's; Triple). ×660. FIG. 18. Sporangiospores with unexpanded nuclei (F.A.A.; Buffer). ×900. FIG. 19. Metaphase, anaphase, and telophase of mitotic division in young coenozygote (Flemming's; Haematoxylin). ×1500. FIG. 20. Expanded nuclei in coenozygote showing central body and chromatic reticulum (Flemming's; Triple). ×1500.

zygospores, although he points out that it is impossible, on the basis of gross examination, to distinguish between living and dead zygospores. During the course of this study a large number of zygospores of *Sporodinia* were germinated and much information concerning the details of this process accumulated. This will be presented in a subsequent publication. There is very strong evidence that in artificial culture at least the zygospores remain viable for only about a month after their formation, and the greatest number of germinations have been obtained from zygospores about two weeks after the formation of the exospore. At this time it was frequently possible to obtain 100 per cent germination of zygospores isolated under sterile conditions and kept uncontaminated by other organisms. After this critical period has passed, the percentage of germinations fall off rapidly and it has proved impossible to germinate spores over six weeks old. For this reason, it has been assumed that spores over this age are probably no longer viable, although Blakeslee's statement concerning the impossibility of distinguishing living from dead spores externally is fully confirmed. Since many of Keene's data were drawn from spores over six weeks old, it appears probable that she was at times dealing with dead spores, and that certain of the peculiar chromatic bodies which she describes in these "mature" spores were, in reality, decomposition products.

Approximately two weeks after the deposition of the exospore the spore contents become vacuolate and much of the reserve substance present disappears. The nuclei assume the expanded condition, but remain in the peripheral regions of the spore and mitotic divisions commence. A certain amount of swelling of the spores takes place, and externally this stage may be recognized by the mottled appearance of the exospore. By the time the oil reserve has been completely absorbd, the endospore and exospore are ruptured at a point usually close to the median line of the spore and a thick germ tube is pushed out (fig. 15). Mitotic divisions take place rapidly as this tube develops, but they differ in no way from those seen in the vegetative mycelium. Growth of the tube is fairly rapid and in a moist atmosphere it may in the course of 48 hours reach a length of 5 centimeters. After the initial period of growth is over, the tube develops a series of dichotomous branches which in turn produce terminal sporangia. If the primary tube is injured or encounters any obstructions several secondary tubes may be developed, and growth of these proceeds until all the reserve contents of the spore have been utilized. In the growing tubes most of the cytoplasm is aggregated near the tips while the older portions of the tube quickly become vacuolate and develop pseudosepta of the same type as those in the asexual sporangiophores. Harper's account (12) of the development of the vegetative sporangia is fully confirmed by a study of both vegetative and germ sporangia. No peculiarities of mitotic division, and no evidence of nuclear

fusion in either germ tube or germ sporangium have been observed. The spores of the germ sporangium are multinucleate and give rise to homothallic mycelia.

From a consideration of the accounts of Dangeard, Leger, Moreau, Lendner, Keene, and Ling Young the conclusion is reached that nuclear fusions occur in the early stages of zygospore formation. All these authors affirm the difficulty in distinguishing nuclear fusion from nuclear division and have shown that only a slight size difference distinguishes fused from unfused nuclei. Lendner alone has been able to demonstrate typical fusion nuclei, but as pointed out above his interpretation may be questioned. Ling Young has indicated that there is apparently no essential difference between nuclear behavior in azygospores, where nuclei of only one potentiality are present, and in zygospores where presumably there are nuclei of two potentialities. Gruber has not seen any fusions whatever nor has he reported any nuclear degeneration. The present study indicates that the size difference in nuclei at various stages of zygospore formation and also at other stages in the thallus may be correlated directly with the state of activity of the cytoplasm. Expanded nuclei in active cytoplasm always appear larger and more chromatic than unexpanded nuclei in senescent cytoplasm. This situation has not been elucidated in previous studies on the Mucorales. Such a concept offers a very logical explanation of the situation described by Dangeard, Keene, and Ling Young, in which nuclei of two types are present during the young zygospore stages, for certain supernumerary nuclei may, just before degeneration, enter the unexpanded phase. If this hypothesis is valid, then in reality no investigator has ever encountered true karyogamy in *Sporodinia*. That such a situation may be true seems plausible in the light of facts garnered from a study of other members of this group where karyogamy is a clearly defined process. The question therefore arises whether karyogamy regularly occurs in *Sporodinia*, or whether the stimulus of plasmogamy is sufficient to induce an apomictic development and germination of the zygospore. In the present study no indication whatsoever of nuclear fusion or reduction division at any stage in the life cycle has been encountered.

The fact that the expression of sexuality in *Sporodinia*, manifested by the production of zygospores, is very largely dependent upon environmental factors as shown by the researches of Blakeslee (2) and Robinson (24) indicates that the sexual potentiality of this species is not particularly strong when compared with such other homothallic mucors as *Zygorhynchus dangeardi* and *Mucor genevensis* in which zygospore production occurs regularly under almost any environmental conditions. This situation, coupled with the fact that undoubted karyogamy has not been conclusively demonstrated, may further indicate that in *Sporodinia* the zygospore no

longer represents a truly sexual spore but is only a highly specialized type of asexual reproduction retaining externally the ancestral zygosporic form. Such an interpretation requires the assumption that homothallism of this type is a derived condition. The work of Burgeff on *Phycomyces* and Krafczyk (15) on *Pilobolus* has emphasized the fact that the homothallism shown by certain isolates of these genera results from a heterocaryotic condition in the mycelia and depends for its expression on the balance existing between nuclei of opposite potentialities present in any given mycelium. Such homothallism is quite different from the genic homothallism of *Zygorhynchus* and some species of *Mucor* in which the mycelium is homocaryotic and the homothallic condition arises from self-fertility rather than from a balance between nuclei of different genotypes. Furthermore, experiments carried out by Ling Young (18) on mycelium regenerated from the suspensors and young progametangia of *Sporodinia* showed that this mycelium was both homocaryotic and homothallic and was capable of producing normal zygospores, thus indicating that in the initials of the sexual organs there had been no localization of opposed sexual tendencies. Since it has been shown (8) that well-defined karyogamies occur in such species as *Zygorhynchus dangeardi* and *Absidia spinosa* which show this same homocaryotic condition, it seems not unreasonable to suppose that in *Sporodinia* the situation has been carried one step further, and that nuclear fusion has become obsolete. Such a simplification of the life cycle might be expected in a highly specialized semi-parasitic species of this type. The development of the zygospore is then explainable upon the basis of habit derived from the parental homocaryotic form which, in turn, was derived from an originally heterocaryotic, heterothallic condition by the appearance in some strains of a condition of self-fertility in the nuclei. Additional evidence for this situation might be deduced from Lendner's (17) report of a reduced number of karyogamies in a strain of *Sporodinia*, although it has previously been suggested that his conclusions may have resulted from the incorrect interpretation of an artifact. A reduction in the number of karyogamies, from a condition of multiple fusions as seen in *Zygorhynchus* to a condition where only two fusions occur, such as Lendner reports for *Sporodinia*, may well be interpreted as an intermediate step in the progression towards a complete loss of karyogamy. It is perfectly plausible that the nuclear condition may not be stable throughout all the races of the species, and that the material used in this study represents a more advanced condition than that present in Lendner's material.

It is evident that a number of strains of *Sporodinia* from different localities must be investigated in order to ascertain whether the nuclear condition is in a transitional state or whether it has reached a uniform development in a wide-range of isolates. These isolates are unfortunately not

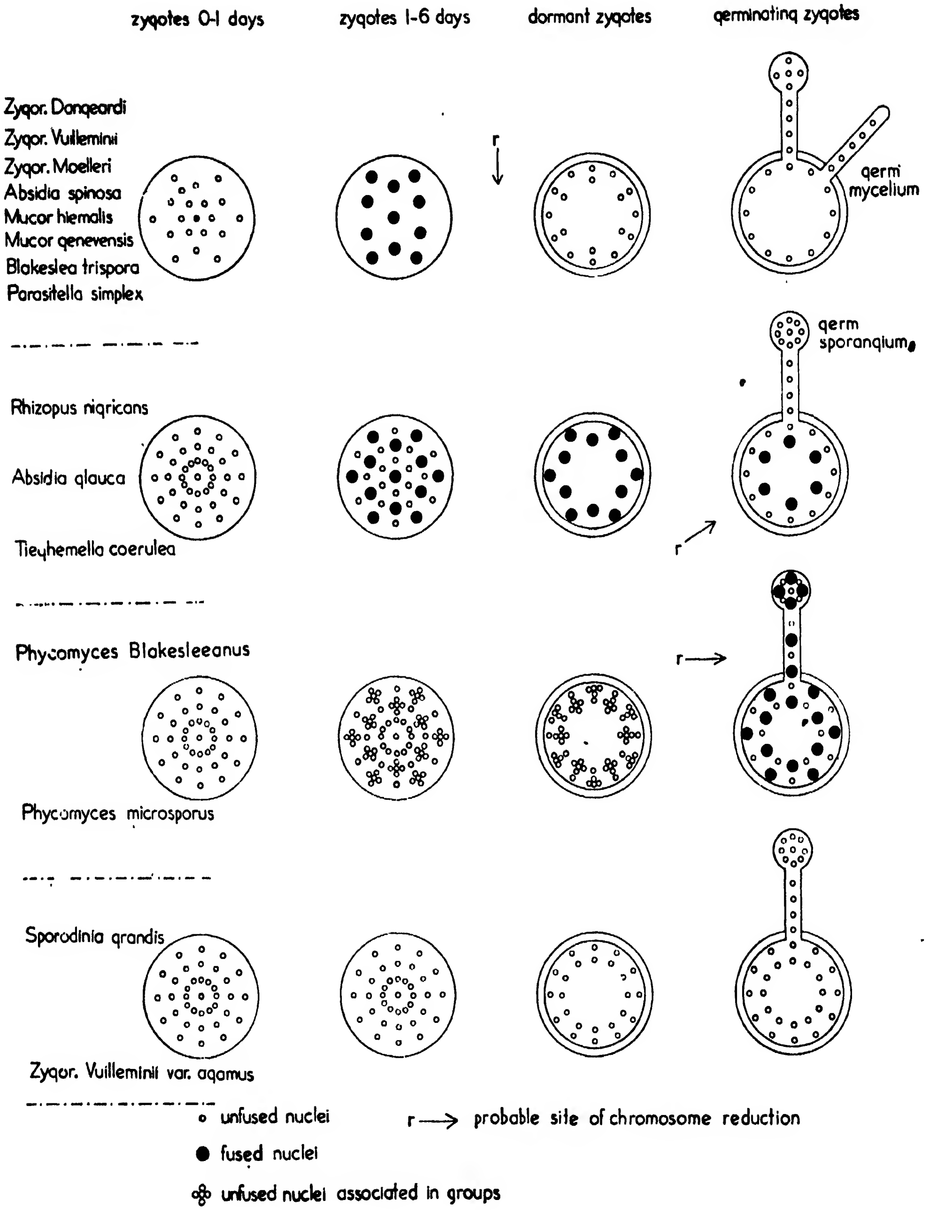

FIG. 21. Diagrams illustrating the probable nuclear condition in the zygotes of the 15 species included in this study. The geometric arrangement of the symbols has no significance except to indicate the approximate distribution of the nuclei at various stages. The arrows indicate the stages at which meiotic divisions presumably occur. The four time intervals given are arbitrary and may vary under different conditions, but merely represent the average intervals at which conspicuous nuclear phenomena occur in the four groups.

available at present. Until undoubted nuclear fusions can be demonstrated, it appears that the development and germination of the zygospores in my material of *Sporodinia* is of an apomictic nature, and in this case plasmogomy furnishes the necessary stimulus to further development ordinarily supplied by karyogamy. The zygospore must then be regarded as a vestigial structure representing the last evidence of an obsolete sexuality.

DISCUSSION

A consideration of the fifteen forms included in this study indicates that four general patterns of development occur. These patterns are illustrated diagrammatically in figure 21. It is possible that further investigation of other species in the order will reveal that all the forms may be grouped according to these general formulae. It is equally probable that still other conditions may exist, or that intergradations between these four basic patterns can be demonstrated. In the species studied here intergrading forms were not observed. Some doubt may arise concerning the correct interpretation of the pattern illustrated by *Sporodinia grandis* and *Zygorhynchus vuilleminii* var. *agamus*. It is evident that the behavior patterns of the nuclei of these forms are not homologous and have arisen in completely different ways. The apomictic development of *Sporodinia* apparently represents the loss of the sexual function of the nuclei, with physiological differences in the plasma, under the influence of external conditions, sufficient to produce a pseudosexual reproductive mechanism. This type of pattern in *Z. vuilleminii* var. *agamus* on the other hand represents a degeneration from the earlier sexual condition caused by a suppression or loss of function of the nuclear complement of one gametangium. That this is an advanced rather than a primitive condition is implied by the fact that on those rare occasions when both gametangia function in zygospore formation the nuclear pattern of the zygote is of the same type as that in the species. It is to be expected that in future work on this problem this fourth category of nuclear behavior will serve as a repository for species in which the loss of sexuality has arisen in several ways, and in this respect it must not be thought of as a natural grouping.

Much further work must be done before any valid conclusions can be drawn on the evolutionary course of these patterns. From this preliminary survey it appears that the *Rhizopus* pattern may have arisen from the *Mucor* pattern by a restriction of karyogamy to certain favored nuclei and a deferment of the meiotic process until the time of germination. This condition is carried further in the *Phycomyces* type in which an association, but no fusion, of nuclei occurs early in the formation of the zygote, the fusion being delayed until the time of germination where it is still restricted to favored nuclei. This condition also appears more specialized than the *Rhizopus* type

in that all the fusion nuclei do not undergo meiosis in the first generation, and that certain nuclei apparently persist throughout the life cycle without undergoing karyogamy. This persistence of unfused nuclei, correlated with only partial meiosis and possible degeneration of fusion nuclei, may indicate the origin of the *Sporodinia* type in which karyogamy is apparently obsolete and unfused nuclei regularly persist throughout the life cycle. These specializations in nuclear behavior can be correlated with a general increase in the size and complexity of the thallus as well as with an increasing tendency for the reproductive structure to be borne upon specialized zygosporic hyphae or zygophores rather than upon apparently undifferentiated vegetative mycelia. Further conclusions do not seem warranted without additional information on transitional types.

It will be noted that the concept of expanded and unexpanded nuclei is of great importance to the interpretation of nuclear behavior in the various stages of the zygote. This concept is equally applicable to fused and unfused nuclei. The numerous reports of nuclear degeneration at various stages in the life cycle, particularly during the dormant periods of the zygospore, are in large part traceable to the failure to distinguish between true nuclear degeneration and the assumption, by persistent nuclei, of the unexpanded phase during periods of cytoplasmic inactivation. In future investigations this distinction should be borne clearly in mind.

The results of this study indicate certain problems which must receive consideration in the future. The most important of these is obviously a further investigation into the process of meiosis in these forms. As yet our knowledge of chromosome reduction is confined to Burgeff's brief discussion of the phenomenon in *Phycomyces blakesleeanus* and the present report of prophase configurations in *Absidia spinosa*. The importance of an understanding of the nuclear patterns described here to phylogenetic studies in this order makes imperative the investigation of other species from the same standpoint. In connection with the apomictic development of the zygospore of *Sporodinia* an investigation of such other semi-parasitic homothallic genera as *Dicranophora* and *Spinellus* may indicate a similar condition. The present study has only partially clarified the nature of the chondriome in the Mucorales, and the origin of the oil plastids and their possible connection with mitochondria and nuclei remains obscure. It is hoped that a further study of these critical phenomena, by the use of more precise techniques, may be instituted in the near future.

SUMMARY

1. The nuclear condition throughout the life cycle of fourteen species and one variety of the Mucorales has been investigated by the use of several fixing and staining techniques not previously applied to this group. The

results obtained with these new methods are compared with those obtained by the usual techniques of fungus cytology.

2. Nuclei are shown to exist in two conditions at various stages in the life cycles. Expanded nuclei occur at all stages where the cytoplasm is in an active state. Unexpanded nuclei are present wherever cytoplasmic activity has slowed down or ceased. The failure to recognize the significance of these two nuclear phases has accounted for several erroneous interpretations in previous investigations.

3. Four patterns of nuclear behavior are described in these 15 forms. In the *Mucor* type all functional zygospore nuclei undergo karyogamy followed by immediate reduction division prior to the onset of dormancy in the zygospore. In the *Rhizopus* type only a portion of the nuclei in the zygospore fuse and the supernumerary nuclei degenerate before the rest period sets in, while reduction division is delayed until the germination of the zygospore. In the *Phycomyces* type the nuclei associate in groups in the young zygospore and persist in this association until the germination of the zygospore. A partial fusion of the nuclei present occurs just prior to germination, but the unfused nuclei do not degenerate. Reduction division of some of the fusion nuclei occurs in the germ tube and the developing germ sporangium, but some of the apparently unreduced diploid nuclei enter the sporangiospores of the germ sporangium. These presumably pass to the next generation in the unreduced condition. In the *Sporodinia* type nuclear fusion apparently does not take place at any stage in the life history and development of the zygospore is apomictic.

4. The possible course of evolution of these patterns is discussed briefly.

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BULLETIN OF THE TORREY BOTANICAL CLUB

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DEVELOPMENT OF SPORELINGS IN THE LEJEUNEACEAE*

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The observation of the sporeling stages of plants has long been of interest not only because of the information concerning the differentiation and specialization of the tissues involved, but also because of the phylogenetic implications which might be derived from these patterns of development. The literature devoted to this sort of observation in higher plants is rather large and well known and no attempt will be made to review it at this time.

The Bryophyta, particularly the leafy Hepaticae, afford an especially stimulating field for studies of this nature. In the first place, our information regarding the ancestral forms of this geologically ancient group of plants is very fragmentary, and these developmental stories give promise of indicating relationships between the modern groups. In the second place, the various tissues of the leafy liverworts are relatively less complex and the mass of cells involved much smaller, so that the various steps in the developmental story can be studied more clearly—often without making sections. Unfortunately, too little attention has been given to studies of this sort, for at the present time our information concerning the sporeling among the leafy liverworts is very meager.[1]

In working over collections from Central America, sporelings were found in several genera of the Lejeuneaceae. They appear to be of special significance, first, because no examples of sporelings with leaves have previously been reported for this largest and most elaborate family of the leafy liverworts, and secondly, because the basic pattern of development is not uniform throughout the group. The several patterns are described below.

1. LOPHOLEJEUNEA SAGRAEANA. The plants of this species form brownish or blackish green mats on trees and wood. The stems may reach 2 cm. in length, the leaves are ovate and rounded-entire, to 0.75 mm. long and 0.55 mm. wide, with the lobule rather small, but conspicuous and strongly inflated. The underleaves are reniform and undivided.[2] The sporelings[3] were mostly 0.2 mm. long. The four or five distinct leaves were deeply pigmented with brown, as are those of the parent plants. They were identified as spore-

* This work was done during a study of tropical American Hepaticae made possible through a John Simon Guggenheim Memorial Fellowship 1941–1942.

[1] For a summary see G. Chalaud in Verdoorn, Manual of Bryology, chapt. 4, 1932.

[2] See Evans, A. W. Bull. Torrey Club 34: *pl. 3, no. 10–20.* 1907, for figure.

[3] Sporelings were found both in the material collected by Steere and Lundell, no. *8060* (Univ. Michigan) in Petén, Guatemala, and by Dodge, Steyermark & Allen no. *16946* (Missouri Bot. Garden), in Panama. All of them were in approximately the same stage of development.

lings rather than developing gemmae because of the presence of the granular exospore which was still conspicuous over the basal part, a feature which according to Goebel[4] is characteristic of sporelings but is absent in gemmae.

No filamentous protonemal stage is formed. Mature spores have not been described for the genus, nor were they found in the material, so that we do not know whether germination begins while they are yet in the sporangium or after they are shed. An early stage in germination showed the presence of two walls more or less at right angles to one another within the exospore (fig. 1, A–C). Divisions within some spores ceased at this point but in others it continued until a somewhat larger, often bulging mass of cells—perhaps ten—was formed (fig. 1, D–E). A rhizoid is often produced from the basal cell (fig. 1, A–C). The growth of the leafy plant is initiated by the activities of the other three cells (or the cells at one end if there are more than four), which probably immediately form an apical cell with three cutting faces. Remnants of the exospore were never observed on the cells of the new leafy plant, which indicates that it breaks through the spore coat, or that the exospore is stretched so thin that it can no longer be observed.

The first leaves, the *primary leaves,* are very small, ovate and plane, each one increasingly larger than the preceding (fig. 1, B–D). In most of the sporelings examined three such leaves were found, but occasionally there were more. Usually the fourth leaf formed is many times larger than these, is saccate and bilobed, with the lobule nearly as large as the leaf (fig. 1, C and D). This saccate leaf is the characteristic *juvenile* leaf of the Lejeuneaceae and is to be found also in *Frullania* and *Porella*[4] and no doubt in certain other of the leafy liverworts. This saccate leaf is the earliest indication of the formation of the watersac so characteristic of the mature leaves (compare fig. 1, C and D, with 1, F).

Concurrent with the formation of this juvenile leaf the first recognizable underleaf is developed (x in fig. 1, C). This juvenile underleaf is narrowly lanceolate, while that of the mature plant is reniform.

The stem continues to form leaves and underleaves of the juvenile type for some time. Unfortunately, none of the plants was sufficiently large to show the transition from the juvenile to the mature condition, but this change is no doubt a gradual one with the lobe becoming larger in each successive leaf formed, since occasionally on depauperate stems or branches of mature plants some of the leaves may be much reduced (juvenile), nearly as small and of a similar outline, and here the transition to the mature condition is gradual.

This type of sporeling has not previously been observed in any of the Lejeuneaceae. The initial stages in the development of the spore, that is, the formation of several cells within the expanding exospore, is similar to the

[4] Goebel, K. Organographie der Pflanzen 2: 906–907. ed. 3. 1930.

condition described by Goebel[4] for *Pellia, Porella, Frullania,* and *Lepidolaena* (*Polyotus*). The young leafy stems are almost identical with those of *Frullania dilatata* in habit, as well as in the character of the primary leaves, the underleaf, and the juvenile leaf which Goebel described and figured. It differs in that in *F. dilatata* the number of cells formed within the exospore is at least eight, usually many more, while in *Lopholejeunea Sagraeana* the number is very often four and never more than eight or ten.

The sporelings described by the earlier writers as indicative of the sort to be expected throughout the Lejeuneaceae are not of the type just described for *Lopholejeunea,* but to some extent do agree with the form described next.

2a. STICTOLEJEUNEA KUNZEANA (?). Mature stems of this species may reach 5 cm. in length, are green tinged with brown, and are usually copiously pinnately branched. The rounded-ovate leaves average 1.4 mm. long and 0.85 mm. wide, the small watersac is narrowly cylindrical in form, and the underleaves are broadly orbicular and undivided. Specialized cells, the ocelli, are scattered throughout the leaf, underleaf, and perianth, and are diagnostic for the genus. The sporelings[5] were abundant among the leaves of the parent material and on *Plagiochila* growing intermingled with it. There were seventy-five or more, all of them approximately the same age and probably from the same sporangium. Unfortunately the mature spores could not be found, nor have they been described previously.

There is formed on germination a flat, linear protonema, one layer of cells thick, two cells broad and usually four cells long. This eight-celled body is 65–80 μ in length and may have been formed by an apical cell with two cutting faces (fig. 2, A–G). Sometimes there are irregularities in the developmental pattern, such as the occurrence of one broad basal cell instead of the usual two (fig. 2, A), or a failure of one or two cells of the pairs to develop so that an irregular filament is formed (fig. 2, C and F), or occasionally the formation of additional cells so that the filament is more than two cells wide in places (fig. 2, G). The development of this eight-celled filament usually takes place within the granular exospore wall, which becomes stretched to many times its original size. The markings are present to a greater or lesser degree on all of the protonemata. However, there were a number of examples in which much of this wall had been shed and remained at the base of the filament (fig. 2, A and B). The markings were present even on these.

Growth of this linear body ceases when the eight-celled stage is reached and growth of the leafy shoot at right angles to the protonema is initiated through the activities of a new apical cell. It develops either at the tip of the

[5] The mature plants belong to that complex of forms more or less intermediate in character between typical *S. squamata* and *S. Kunzeana,* which is so often to be met with in Central America. The collection was made by Dodge & Goerger, No. *9905,* in Costa Rica and is in the Missouri Botanical Garden.

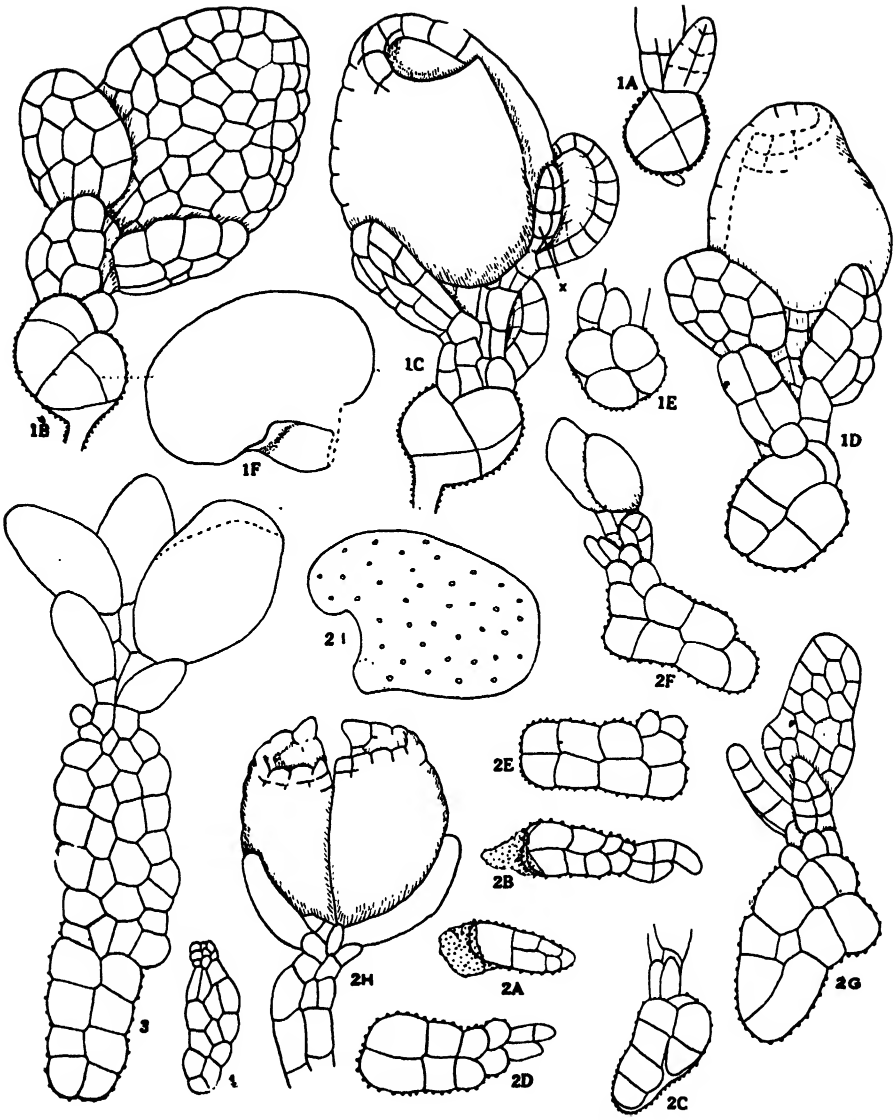

FIG. 1. *Lopholcjeunea Sagraeana.* 1A. Early stage of spore germination showing the divisions within the spore, the first leaf, and a portion of the stem. × 300. 1B. A sporeling with three primary leaves and a juvenile leaf; dorsal view. × 300. 1C. The same, showing the first underleaf (X) and the sac-like juvenile leaf in more detail; ventral view. × 300. 1D. Another sporeling; dorsal view. × 300. 1E. A sporeling in which many cells have been formed within the exospore. × 300. 1F. A mature leaf; ventral view. × 30. FIG. 2. *Stictolejeunea Kunzeana* (?). 2A, B. Young protonemata with the discarded exospore. × 300. 2C. An irregularly formed protonema; × 300. 2 D–F. Typical eight-celled protonemata showing the first cells of the leafy stem. × 300. 2 F–G. Sporelings with protonemata and the three primary leaves. × 300. 2 H. A sporeling with remnants of the protonema, two primary leaves, and two sac-like juvenile leaves; ventral view. × 300.

protonema (fig. 2, B–D, H), or on either side between the third and fourth cells (fig. 2, E–G), and we may assume that if not at the beginning, at least very soon it has three cutting faces. The first, second and third leaves are small, each progressively larger than the preceding, and plane (fig. 2, F–G), and in no way indicative of the mature leaf (fig. 2, I). These are the *primary* leaves. One or more ocelli, so characteristic of the mature leaves and underleaves, are produced in the largest of these leaves (fig. 2, G). Very often at about this stage a rhizoid can be observed at the base of the leafy shoot. The fourth leaf formed is of a different sort and is a juvenile leaf typical for Lejeuneaceae. It is much larger than the preceding primary leaves and is definitely saccate, with the lobule nearly as large as the lobe. A sporeling with two such leaves is shown in figure 2 H. It is at about this stage of development that recognizable underleaves should also become noticeable but only one plant had reached this stage of development and the underleaf was not evident. Unfortunately none of the plants was large enough to show further stages in development.

2b. A sporeling with a somewhat similar yet more elaborate protonemal phase was found among plants of *Archilejeunea porelloides* collected by Spruce in the Amazon Valley. It is *not* a sporeling of that genus. It seems to be of the same type which Goebel[6] has figured for an unknown South American species of the Lejeuneaceae. His plants had not yet developed the leafy stem. An eight-celled linear protonema identical with that described above for *Stictolejeunea* developes from or in, the spore, for the granular exospore markings are plainly visible. In addition there is a much larger plate of cells nearly twice as long as the basal portion and four cells wide, developed at the end. The leafy stem arises at the end of this body (fig. 3). The development of this leafy stem is also similar to that described above. The apical cell with three cutting faces cuts off two or three leaves of the *primary* type and after that a *juvenile* leaf of the Lejeuneaceae type. The next leaf on our specimen was a primary leaf.

2c. Goebel[7] has also described and figured the developmental stages of the sporeling of LEJEUNEA CAVIFOLIA (a genus belonging to the *Schizostipae*, the section with divided underleaves). The mature thallus of this species is somewhat similar to that described for *Stictolejeunea*. Its development from the spore comes about in either of two ways. The spore elongates and divides and redivides, thus forming a four-celled germ tube, in the terminal cell of

[6] **Goebel, K.** op. cit. 907. *fig. 972.*

[7] **Goebel, K.** loc. cit.; and Flora **72:** *pl. 1, fig. 18.* 1889.

2I. A mature leaf. × 30. FIG. 3. A sporeling of an undetermined South American species of Lejeuneaceae, showing the eight-celled filamentous stage similar to that of *Stictolejeunea*, the longer and broader filament developed from it, and the young leafy stem. × 300. FIG. 4. A protonema of *Lejeunea cavifolia*, the leafy stem beginning to develop. (Fig. 4 after Goebel.)

which an apical cell with two cutting faces developes. This apical cell gives rise to the linear protonema two or three cells broad. Or, after the elongation of the spore and one division, the apical cell which is to form the protonema developes in one of the cells, while the other cell divides longitudinally. This type of protonema, which is also characterized by granular markings (fig. 3), differs from that of *Stictolejeunea* in that it is made up of more cells and is less regular in plan (compare fig. 2, A–C, with fig. 4). Goebel concluded from his observations of this small linear protonema of *L. cavifolia* and the larger one of the undetermined South American Lejeuneaceae genus, that this general thalloid type was characteristic of all the Lejeuneaceae, an assumption which is incorrect in view of the pattern of development of the sporeling of *Lopholejeunea Sagraeana*. It is of interest to note that the sporelings of *Radula* also have a filamentous protonema.

The discovery of several patterns of development among the sporelings of the various genera of the Lejeuneaceae gives rise to several questions of interest from the morphological as well as phylogenetic point of view.

In the first place, should one call the massive many-celled structure which developes within the exospore wall in *Pellia, Porella, Lepidolaena, Frullania,* and *Lopholejeunea* a protonema? If so, is the flat, linear structure, which also has evidence of exospore on the cells, produced by the spore of *Stictolejeunea* and others, a protonema, and is it analogous or homologous with the initial stages in *Lopholejeunea?* Or are there two patterns of spore development, one without and the other with a protonema? In comparing figures 2, D–H, with figure 3, should all of the thallus body of figure 3 be considered the protonema, since in this example an additional thallus structure occurs between the basal eight-celled thallus with exospore markings similar to that of *Stictolejeunea* and the leafy shoot?

In the second place, are developmental patterns of sporelings of value in establishing ancient or recent relationships?

Since we have so little information concerning the development of the sporelings in the Lejeuneaceae or, for that matter, in most of the families of leafy liverworts, discussions of relationships must be postponed. It would seem however, that such patterns of development, when assembled from a large number of genera, together with other pertinent facts, should contribute significant data.

For the present we must limit ourselves to the conclusion that there are two, perhaps more, distinct patterns of spore germination within the family (even in one section, the *Holostipae,* with entire underleaves). One type, not previously reported in this family, occurs in *Lopholejeunea,* and has also been described for *Frullania* and certain other genera, and could well be designated the *Frullania* type. The other one, in which a flat, one-layered filament or thallus is produced includes several variations:

2a. the *Stictolejeunea* type, in which a small, regular, eight-celled linear filament four cells long and two cells wide, precedes the formation of the leafy shoot;

2b. another type, which must remain unnamed for the present, in which the *Stictolejeunea* type of protonema of eight cells gives rise to a broader and longer secondary thallus at the tip of which the leafy shoot is formed;

2c. and the *Lejeunea* type, in which a small, *irregular*, linear protonema one cell thick, two and three cells broad, and more than four cells long precedes the formation of the leafy shoot.

In the progressive development of the leafy stem three distinct phases are evident. In the earliest of these, few-celled, ovate, and plane *primary* leaves not accompanied by underleaves are formed. These are followed by larger, characteristically saccate, *juvenile* leaves in which the lobule and lobes are approximately the same size, and are typically associated with small, lanceolate underleaves. These are soon followed by small leaves and underleaves of the adult pattern which, in the succeeding series formed, are increasingly larger until the mature size is reached.

I wish to express my appreciation to Dr. A. W. Evans for his assistance in the interpretation of the material and for his helpful criticism; and to the Botany Department of Yale University where working facilities were provided.

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SYMMETRY STUDIES IN SAGINA

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Numerical variation in flower parts has been studied in a relatively small number of plant genera. The present work was undertaken to investigate certain tendencies of variation and reduction found in the family Caryophyllaceae. The genus best known from this standpoint is *Stellaria,* in which many trends have been shown.

Sagina was chosen because one species, *S. procumbens* L. possesses flowers characteristically in fours, although the other members of the genus normally have the parts in fives. Data on the number of flower parts, inflorescence, and on the habits of growth of *S. procumbens* have been assembled in the present investigation.

REVIEW OF LITERATURE

The genus *Sagina* (L.) was established by Linnaeus, containing *S. procumbens.* It has been confused from time to time with *Spergula* and *Arenaria.* Both *Spergula* and *Arenaria* normally have a full complement of petals, while *Sagina* has always been conspicuous for the absence of some or all of the petals.

Eichler (1878), Pax (1889), and Warming (1904) discuss the stem and inflorescence in their treatment of the Caryophyllaceae. Wydler (1847, 1851) and Wichura (1844, 1846) engaged in a lively controversy over the branching and phyllotaxy of the group, and established many facts concerning family characters. Schoute (1932a, 1932b, 1936, 1938) has discussed the decussation and whorl formation in the group in his treatment of the nature and development of opposite and whorled phyllotaxy. He looks upon whorl formation as a process occurring some time during the growth period, usually in the early stages. That opposite arrangement was of secondary origin was recognized by Church (1904) and Čelakovský (1875, 1902), though the contrary has been also maintained (see Sprague, 1925).

The principles of phyllotaxy have remained unchanged for almost a century, and no theory of its intrinsic nature has met with any complete acceptance. The Fibonacci series of Schimper and Braun has not proven inclusive of all phyllotactical patterns known and was attacked by Church (1904), who pointed out its inaccuracy in shoot apices where the divergence is commonly 180 degrees. Davies (1939), however, submitted data on *Ailanthus* to show that the ideal $\frac{3}{8}$ divergence of 137.30′ degrees is closely approx-

[1] The publication of the illustrations is assisted by a contribution from the author.

imated when the shoot is developed, although the divergence is ½ in the bud region.

Although no special literature has been found dealing with *Sagina*, the genus, according to information in current manuals, has been conspicuous for variation in the number of petals. Since all other species possess flower parts in fives, and *S. procumbens* has flower parts in fours, some variation in the other whorls might well be expected.

Schoute (1932a) discusses some of the reasons advanced by authors to account for floral variation, and concludes in favor of the general theory of Eichler (1875) called "originäre Variabilität" to account for variation in plant families and plants with higher floral numbers. That vigorous plants and first-formed flowers tend toward variation may also be explained by this theory.

Schoute also points out that Eichler's theory affords a plausible explanation of the foregoing facts, and criticizes the theory of "dédoublement" (Čelakovský), and other theories of abortion, fission and fusion.

Matzke (1930a) investigated the variation of stamens of a single pure variety of *Stellaria media*, and confirmed the rule that age affects the number of flower parts produced. The same author (1930b) found the floral numbers to be lower in variegated plants. By cutting off the flowers after anthesis, he found an increase in the number of stamens produced by subsequent flowers.

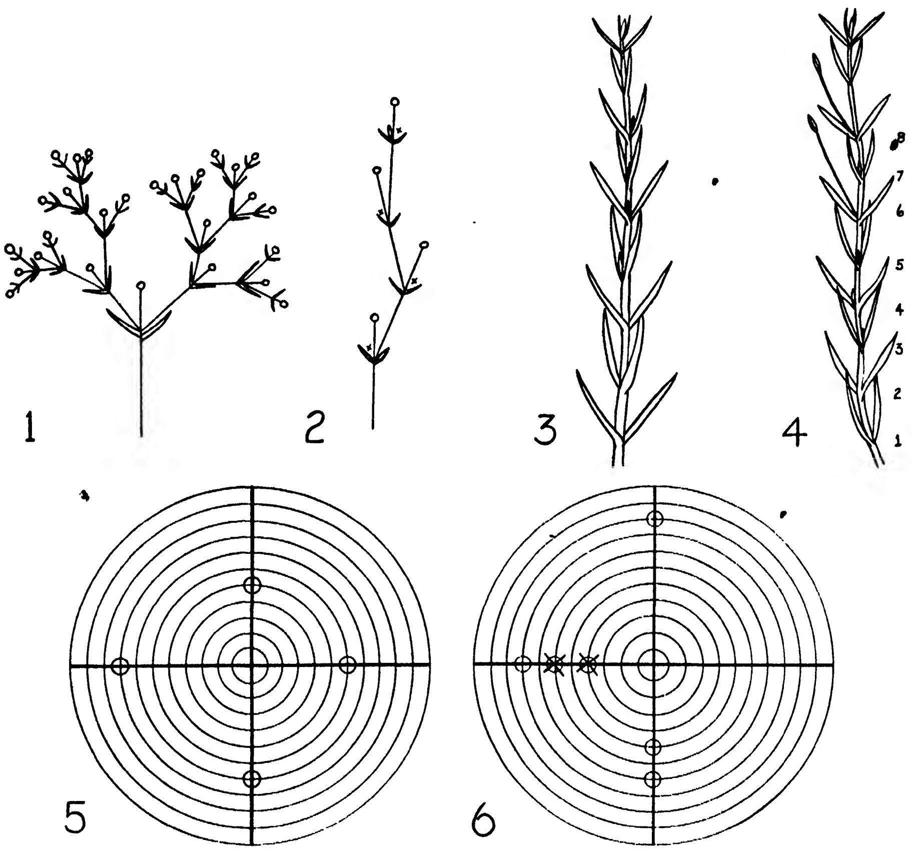
In another paper, Matzke (1932) figured floral diagrams of 4652 flowers of *Stellaria media neglecta* var. *typica.* He found that stamens were lost at definite floral positions, and that symmerical patterns were more common. Reduction of flower parts in certain positions definitely limited the appearance of symmetrical patterns.

The plants of *Sagina* have been characterized as low matted herbs with decussate leaves and glabrous stems and leaves. The inflorescence is cymose or a reduced stage of that type. Graebner (1919) speaks of the inflorescence as composed of "armblüthige Trugdolden." The presence of terminal flowers in the genus is indicated by most authors: Eichler, Pax, Warming, Britton and Brown (1913), Robinson, and Fernald (1908), and Graebner. Eichler's figure labeled "Terminalblüthe" is adopted by Pax (1889) in his treatment of the Caryophyllaceae in Engler and Prantl's ***Die natürlichen Pflanzenfamilien,*** and by Warming. Rydberg (1932) describes the genus in North America as having axillary flowers only.

The floral formula given by Eichler is K4, C4, A4 or 8, and G4 for *S. procumbens.* In his figure, Eichler shows 8 stamens for *S. procumbens.* Pax includes a top view of a flower from Baillon's work, which shows 8 stamens in obdiplostemonous arrangement. The American manuals concur in assigning 4 stamens to *S. procumbens,* and Hegi (1906) mentions 4 stamens for

the species, while Graebner describes a subgenus *Saginella* possessing 4 stamens.

In most of these manuals, *S. procumbens* is described as a species without varieties, but Graebner recognizes 21 varieties and subvarieties plus one definite hybrid. The plants used in this investigation are *S. procumbens glaberrima praecox* of Graebner.



FIGS. 1, 2. A typical cymose inflorescence and the uniparous scorpioid cyme derived from it. FIGS. 3, 4. Branches of *Sagina procumbens* showing bud spirals and flower positions discussed in the text. FIGS. 5, 6. Phyllotactical diagrams of figures 3 and 4 respectively.

THE STEM AND INFLORESCENCE

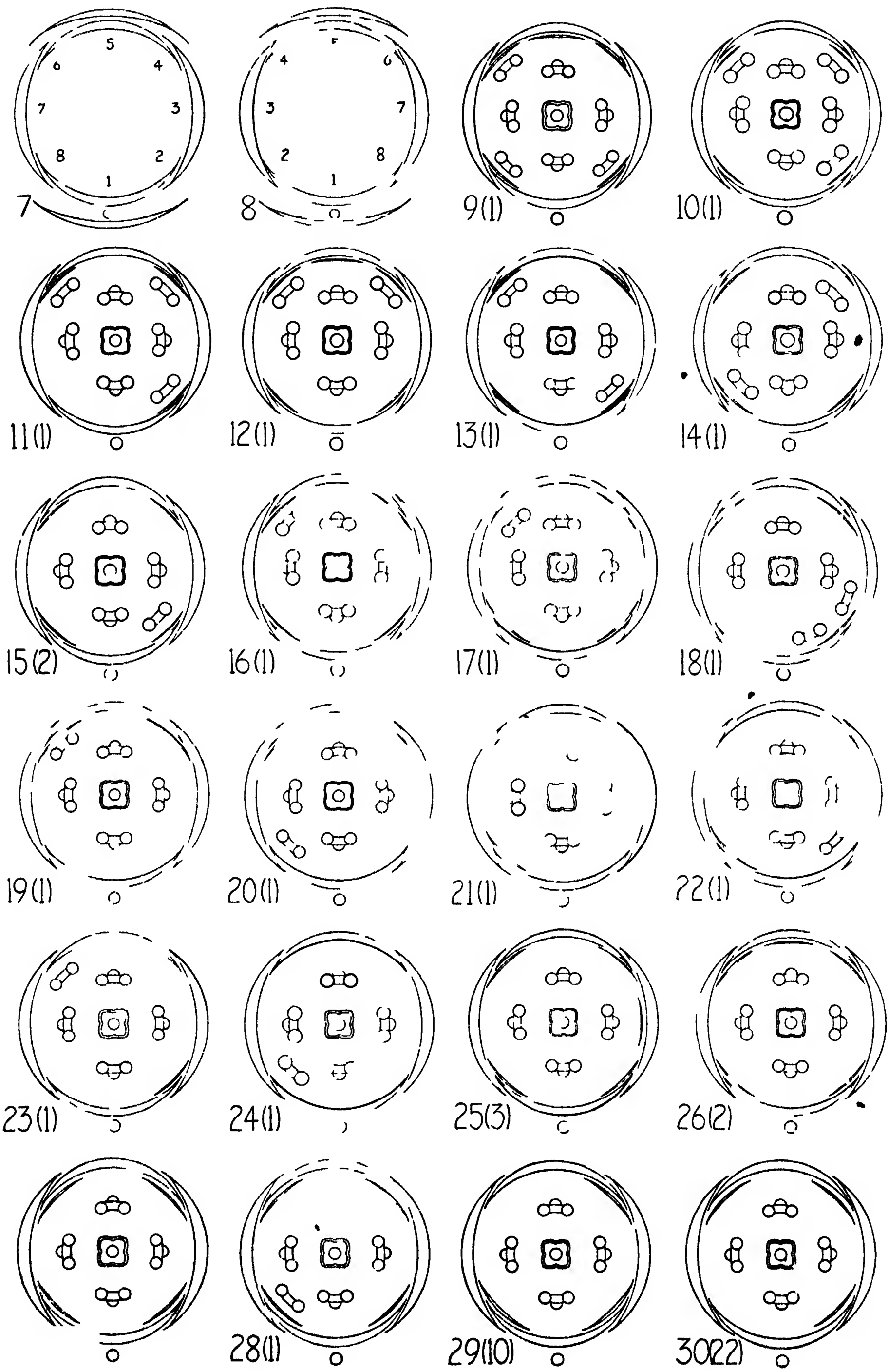
The method of branching was observed throughout the investigation. Difficulty was experienced in studying the production of branches from the basal rosette. The cotyledonary node is quickly drawn to the surface of the soil, and, as more leaves are produced on the axis, the first leaves, being close to the substratum, decay rapidly. The decussate arrangement present in the young seedlings soon gives way to the rosette appearance,

so that the true arrangement is obscured. Germination of seeds of other species of *Sagina* from New York Botanical Garden herbarium sheets was attempted. A few seeds of *S. occidentalis* S. Wats. germinated. In these the primary axis appeared to branch in the same fashion as did the *branches* of *S. procumbens.*

Contrary to the habit of most Caryophyllaceae and some of the species of *Sagina,* the vegetative buds in the leaf axils were present in only one leaf axil of each pair of leaves (figs. 3, 4). These buds formed a spiral which ascended the stem in typical manner. Of 347 branches studied, 150 were left-hand spirals and 176 spiraled to the right, while 21 could not be determined. These nearly equal totals are in essential agreement with results recorded by Matzke in *Stellaria.* Of more than 100 primary axes studied, most could not be determined, but 21 seemed to be in a right-hand spiral, and 9 in a left-hand spiral. Buds were seldom found in the first and second whorls, but usually were present at the higher nodes. Sometimes these lower nodes developed buds at a later date.

The most interesting exception to the perfect continuance of the genetic spiral of buds was the position of the seemingly axially-placed flowers (fig. 4). Here the spiral seems to be reversed in direction for one node, and then to continue in the original direction. The same characteristic appeared whenever flower buds were produced, no matter how many were produced on one axis. Only two of the stems studied did not show this feature. Figure 3 shows a branch with a single spiral of buds, while figure 4 is a branch whose bud spiral is seemingly interrupted by two flower buds. Figure 5 is a phyllotactical diagram of figure 3, while figure 6 is a phyllotactical diagram of figure 4. The crosses mark the position of flower buds. Here the reversion of the spiral can easily be traced.

This character obviously seems to be a function of the inflorescence. Since Warming describes the inflorescence of the Caryophyllaceae as a dichasium passing into a uniparous scorpioid cyme, it is well to interpret these observations in the light of that statement. All that needs to be shown is that this inflorescence is a reduced form of the dichasial inflorescence type. Figure 1 is a diagram of a dichasium with the uniparous scorpioid cyme derived from it in figure 2. The small crosses in the leaf axils show the positions of missing branches. It is quite easy to see that the inflorescence of figure 4 fits into the pattern of figure 2 on a somewhat more reduced scale. Since every flower in figure 2 is a terminal flower, we must also consider those in figure 4 as terminal flowers. Then the main axis in figure 4 must end in the flower at node 6, and the portion which grows further up is a branch from the axil of the right-hand leaf of node 6. The same reasoning would apply to the flower at node 8. In this way the difficulty about the spirals becomes obviated, and the flowers may be interpreted as terminal members of a reduced dichasium.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25(3)
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28(1)
29(10)
30(22)

To check this supposition further, several hundred herbarium specimens of many species of *Sagina* were studied. Nearly 100 specimens of *S. procumbens* from America and Europe failed to reveal any inflorescence type different from that already described. Other species yielded similar results. Among these were *S. apetala, crassicaulis, elliotii, nivalis, decumbens, decumbens* var. *Smithii, ciliata, glabra, maritima, occidentalis, Linnaei,* and *subulata. S. nodosa* appeared to have mostly axial flowers, but some specimens, especially f. *laxa,* in which stems and flower peduncles were long, showed typical cymose inflorescences. This species, *S. nodosa,* differs from the others by producing petals twice as long as the sepals, and in having shoot buds in the axils of both leaves at each node. This last fact coincides very well with the presence of dichasia in this species. The specific name is derived from the nodular appearance of the shoot buds in the axils of each leaf pair.

THE FLOWER

A study was made of the floral organs and their positions, so that general symmetry patterns might be observed. Figures 9–72 show the different patterns found, and the frequencies of each. Those patterns which were mirror images along the vertical axis were kept together and counted as the same, but those images which mirrored along the horizontal axis were kept separate (e.g., figures 33, 34). (The vertical axis is a line running from position 1 to position 5 in figures 7 and 8; the horizontal axis is a line running from position 3 to position 7 in figures 7 and 8.)

The typical flower of the species is figure 27, which has parts in fours with two outer and two inner sepals easily distinguished. Of 325 flowers recorded, only one had five sepals (fig. 72), and only one had three sepals (fig. 71). The sepal number appears to be fairly constant. A consistent relation was found between the main axis and the sepals of the axially-appearing flowers, since one of the inner sepals was observed always adjacent to the main axis. With this in mind, an orientation diagram was constructed in order to fix floral positions. Beginning with the axis and numbering counter-

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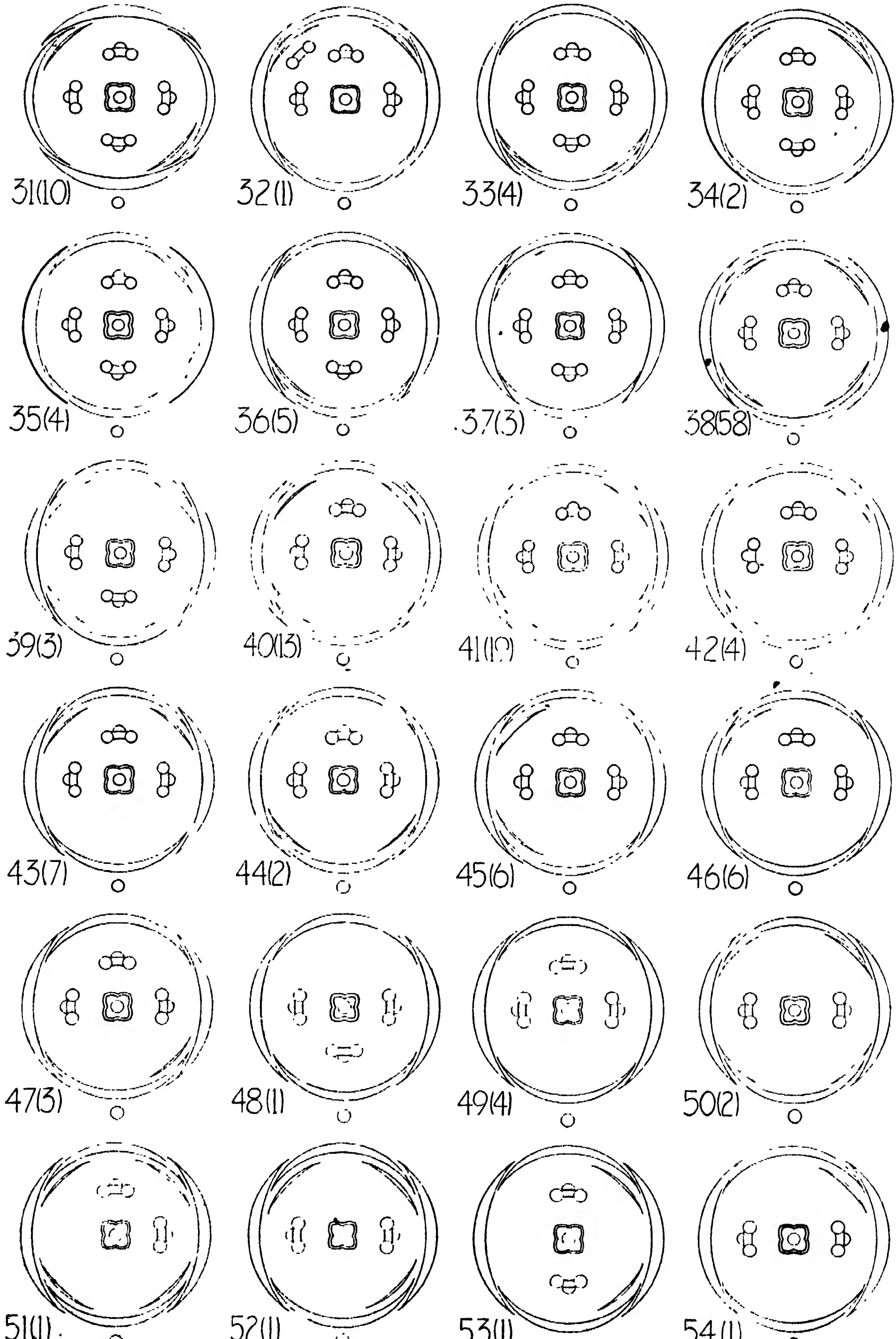
Explanation of figures 7–30

Floral diagrams of *Sagina procumbens.* Figures 7 and 8 are orientation diagrams showing subtending leaves, the axis, and with possible floral positions numbered. The frequency of occurrence of each flower type is given in parenthesis with each diagram. Diagrams are arranged in decreasing order of stamen and petal number.

Number of petals		Figs.
5	Figs.	9, 12, 25, 26
4		10, 13, 15, 16, 27, 28
3		11, 14, 17, 18, 29, 30
2		19
1		20–23
0		24

Number of stamens		Figs.
7	Figs.	9–11
6		12–14
5		15–24
4		25–30


31(10)
32(1)
33(4)
34(2)
35(4)
36(5)
37(3)
38(58)
39(3)
40(13)
42(4)
43(7)
44(2)
45(6)
46(6)
47(3)
48(1)
49(4)
50(2)
51(1)
52(1)
53(1)
54(1)

clockwise, the eight possible floral positions were numbered (fig. 7). Since there is no spiral imbrication of the sepals, the direction of the numbering is purely arbitrary. Figure 8 is included as the clockwise numbering of the diagram, although results in table 1 are based on figure 7 as a reference. The diagrams differ from that of Eichler in that four stamens were found to be typical, rather than eight, and in that the innermost bracts are on the vertical axis rather than the horizontal axis. Figures 7 and 8 show the main axis and the leaves subtending the flower; only the axis is represented in the other figures. Flowers shown in figures 66–70 seemed terminal and so there is no axis shown, but the leaves subtending them are included.

Results in table 1 in the petal section show that considerable numbers of petals disappeared from all of the normal petal positions as seen in figure 27. It can be seen from the data here presented that the possession or loss of petals was by no means confined to any one position. Flowers were found with any or all of the petals missing. The range was zero to five petals (figs. 9, 10, 11, 19, 20, 48). It is possible that because of the small size of the petals, their positions are not strongly affected by spatial relations, nutrition, or balance. Loss of petals may therefore be explained on the basis of Eichler's "originäre Variabilität."

Since positions 1, 3, 5, and 7 are not normal petal positions as shown in figure 27, they are listed in the places where petals might be added (table 1). Since five petals was the highest number found, not more than one petal was ever added to a single flower. Since there were only seven flowers with five petals, no position could be designated as that to which petals are conspicuously added (figs. 9, 12, 25, 26). Six of these flowers were of larger size than usual, and were produced on fewer-flowered plants. Such flowers might have more "available space" and thus might more conceivably produce higher petal numbers.

The number of stamens was also found to have considerable variation but along regular tendencies. Staminal numbers ranged from seven to zero (table 1). It is clearly indicated that only flowers with three or four stamens were frequent in appearance, since flowers with other staminal numbers formed only 7.7 per cent of the total. That the stamens are usually lost from a definite position in the flower is shown by table 1 (stamen section). Of all the stamens missing, 88.7 per cent were dropped from position 1. Of 131

Explanation of figures 31–54

Floral diagrams of *Sagina procumbens*.

Number of petals		Number of stamens	
4	Figs. 38, 39, 50, 51	4	Figs. 31–37
3	40, 41, 52	3	38–49
2	31–35, 42–45, 53	2	50–54
1	36, 37, 46, 47, 54		
0	48, 49		

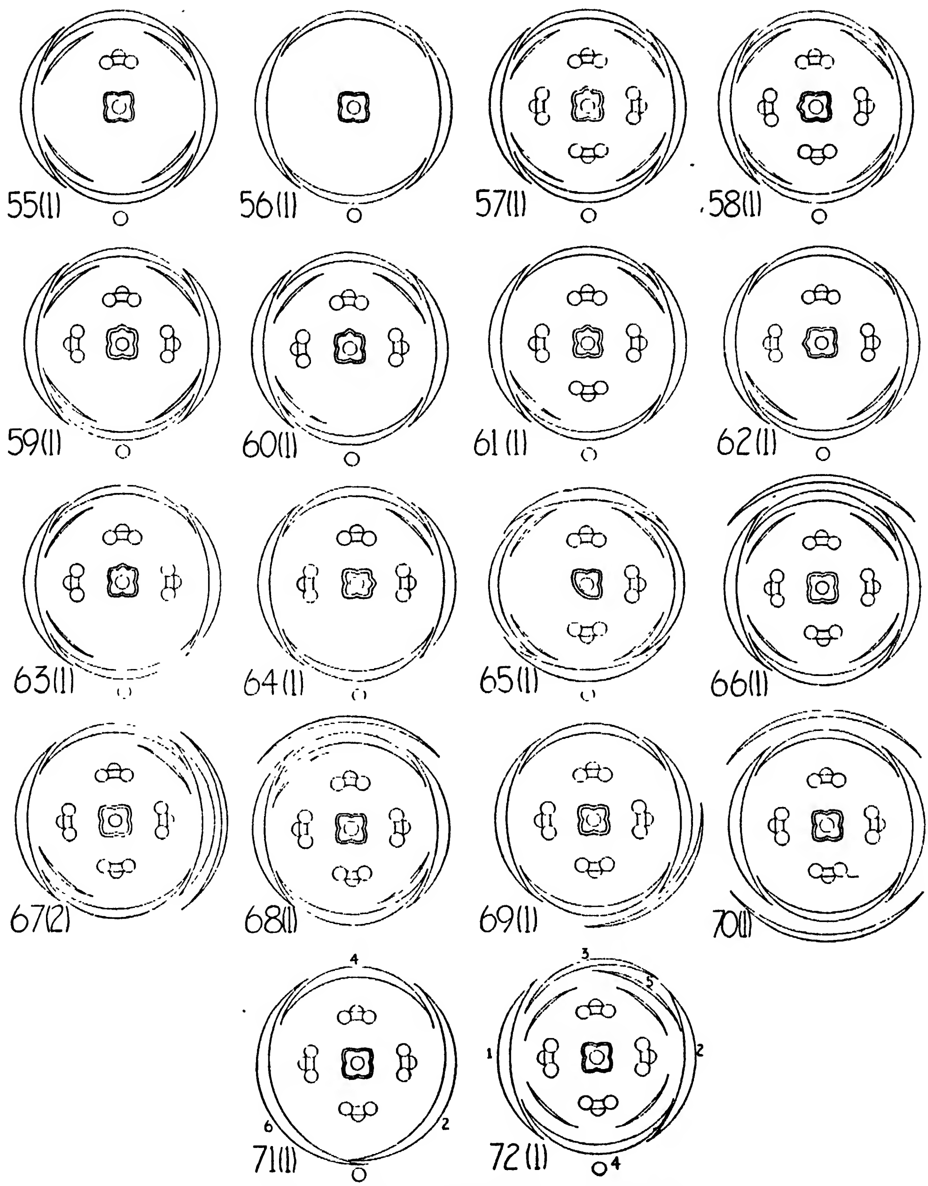
three-stamened flowers, 127 lost their stamen from position 1 (96.9 per cent). As can be seen, this is a position opposite one of the inner sepals. The remaining three-stamened flowers except that shown in figure 65 lost a stamen from position 5, which is also opposite one of the inner sepals. Since there is obviously less space on these radii, and probably in position 1 which is crowded against the axis, there appears to be good correlation with Hofmeister's theory of available space.

TABLE 1. *Frequency of flower parts in S. procumbens with reference to the positions of the flower from which they are gained or lost*

Number of parts		Flowers with parts missing		Flowers with parts added	
Number	Frequency	Floral position	Frequency	Floral position	Frequency
		Sepals			
5	1	1	1	2	0
4	323	3	0	4	0
3	1	5	0	6	1
		7	0	8	0
		Petals			
5	7	2	72	1	0
4	167	4	57	3	3
3	70	6	69	5	4
2	48	8	77	7	0
1	23				
0	10				
		Stamens[a]			
7	3	1	134	2	8
6	3	3	3	4	4
5	11	5	9	6	11
4	169	7	5	8	4
3	131				
2	6				
1	1				
0	1				
		Styles			
5	8	2	0	1	0
4	316	4	0	3	1
3	1	6	0	5	5
		8	1	7	2

[a] Figure 32 makes an apparent discrepancy in the frequency totals here.

Of six flowers with two stamens, five have stamens symmetrically placed (figs. 50–54). There are too few such flowers to permit the drawing of definite conclusions, although they are predominantly symmetrical. Single instances of stamenless and one-stamened flowers were observed (figs. 55, 56).



Floral diagrams of *Sagina procumbens.*

Number of petals	
4	Figs. 55, 57–59, 65, 66, 72
3	60
2	61–63, 67, 68
1	64
0	56, 69–71

Number of stamens	
4	Figs. 57, 58, 61, 66–72
3	59, 60, 62–65
2	
1	55
0	56

Reference to table 1 shows that the extra stamens were not added in any regular manner to the epipetalous radii. Since five-stamened flowers were produced mostly during early flowering, when flowers are largest, an increase in staminal number might well be expected.

Of 325 flowers, all had four styles except eight which had five styles, and one which had three styles. The extra styles are seen to occur in positions 3, 5, and 7, with most of them in position 5, which is opposite the inner sepal on the side away from the axis. It will be noted that all the styles were added to the outer or anterior side of the flower, which is away from the axis, a position which could possibly have more "available space" (figs. 57–64).

DISCUSSION AND INTERPRETATION OF RESULTS

Reasons have already been advanced for the occurrence of apparently axial flowers in *S. procumbens*, and for the loss of petals from all petalous radii. Stamens, however, were lost with definite regularity from position 1. This is the sepalous radius which is adjacent to the axis, so that it is very possible that such a radius would be decreased in area by crowding and pressure of the axis during the developmental stages. As was pointed out, this would agree with Hofmeister's theory of available space. In *Stellaria* also, Matzke found stamens lost from more crowded radii.

Floral reduction is one of the most outstanding features of the Caryophyllaceae, and of the genera *Sagina* and *Stellaria* in particular. This is expressed in a decreasing series of floral numbers. Other factors are also of importance at least in the two genera mentioned. If symmetry, involving architectural balance in the flower, were the major factor in determining floral numbers, a two-stamened flower should be the next most frequent after the four-stamened one, rather than the three-stamened pattern which appeared as a second major type (in the genus *Sagina*).

Matzke reported nine-stamened flowers most frequent after ten-stamened ones in his study of *Stellaria*. On the basis merely of reduction involving radial symmetry, the five-stamened flower or possibly an eight-stamened form should have been next most frequent after the ten-stamened type. Both of these were abundantly produced. Clearly the same condition obtains in both genera. It would be difficult to conceive of a ten-stamened flower progressing to the five-stamened stage in a single step. Thus symmetry relationships seem significant in the orientation of flower parts in *Stellaria* and *Sagina*.

By virtue of its extreme reduction in floral numbers and in the condensation of its primary axis, budding and inflorescence, *S. procumbens* appears to be the most reduced species in the genus *Sagina*, while *S. nodosa* appears least reduced.

SUMMARY AND CONCLUSIONS

1. The inflorescence of *Sagina* shows marked reduction in the cymose type of inflorescence.

2. *S. nodosa* appears to be the least reduced species of the genus, and *S. procumbens* appears most reduced.

3. The loss of petals in *S. procumbens* follows no definite order, but rather conforms to Eichler's theory of simple variation.

4. Four is the common stamen number in *S. procumbens,* and not eight as Eichler's diagram might lead one to suspect.

5. The loss of stamens in *S. procumbens* is largely from one definite position and conforms with interpretations of reduction and of Hofmeister's theory of available space.

6. That the factor of age influences the number of floral parts, as found by older authors, is also true in *S. procumbens.*

7. These tendencies in reduction are in essential agreement with similar expressions in the related genus, *Stellaria.*

I wish to thank Dr. E. B. Matzke for his guidance and help in furthering this investigation.

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THE ESTABLISHMENT OF A WHITE BIRCH COMMUNITY ON CUTOVER PULPWOOD LAND IN NORTHWESTERN MAINE

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Although short-lived, slow growing, and definitely a temporary species, white birch (*Betula papyrifera* Marsh.)[1] is economically a very important component of the second-growth forest of northwestern Maine. In this region white birch was first exploited appreciably as a timber tree about 1880. The wood was used almost exclusively for spools, shoe pegs, shoe shanks, toothpicks, dowels, and miscellaneous wood turnings until shortly after 1910, when increased demands for these articles brought about the use of other hardwoods as substitutes. The available supply of white birch in Maine has dwindled appreciably since about 1930, because of the continued demand for its use in turnings and manufactured wood products as well as its use for pulpwood. Until recently cutting of white birch in western Maine has been haphazard and with little or no concern for the future. The rapid depletion of merchantable stands of this valuable species has emphasized the desirability of applying silvicultural practices which may assure a continued source of supply. To this end, intensive studies of the white birch community are necessary and ecological studies may contribute materially.

Fire has long been known to be the agent primarily responsible for the establishment of the white birch–aspen type in the Northeast. This forest type is practically restricted to burned-over areas (Dana 1909, 1930). With the increased protection against fire of recent years, the type is more limited in area than formerly. Since it would not now seem plausible to assume that fire should be used to promote the development of the birch–aspen type, and thus assure a supply of white birch, it follows that the future supply of white birch will be derived from its present natural reproduction on cutover pulpwood lands where it now occurs as a component of types of communities other than birch–aspen.

Dana (1909, 1930) summarized the occurrence of white birch on unburned areas in the Northeast in the following communities: (1) in pure stand; (2) in northern white cedar (*Thuja occidentalis*) communities of the flat, wet areas in the valleys; (3) in admixture with red spruce (*Picea rubens* Sarg.) and balsam fir (*Abies balsamea*) at the higher elevations in the mountains; and (4) with other hardwoods: namely, yellow birch (*Betula lutea*), sugar maple (*Acer saccharum*), and beech (*Fagus grandifolia*). Westveld

[1] Nomenclature is that of Gray's Manual, 7th Ed., unless authorities are given.

(1930b, 1931) further described the yellow birch–spruce subtype of the spruce–hardwood type in which yellow birch, red maple (*Acer rubrum*), and white birch grow in association with red spruce and balsam fir.

Although as early as 1905, there was some use made of maple, beech and birch in the manufacture of pulp (Hale 1906), it was much later in northwestern Maine that the demand for hardwoods in the pulp industry brought about clear-cutting of the mixed spruce–hardwood forests. As late as 1931 studies on the regeneration of forests on cutover lands refer exclusively to balsam fir and spruce as the pulpwoods (Westveld 1930a, 1930b, 1931). Thus it is that for approximately the past ten years only have extensive areas of mixed spruce–hardwood forests been clear-cut for pulpwood. These cutover areas are now being reoccupied by a young forest, the exact nature of which can only be well known after concentrated studies, preferably phytosociological, have been made locally of the developing communities.

Locally, white birch may appear as the major dominant in these young stands which are then probably to be classified as a phase of the "yellow-birch subtype" of the mixed spruce–hardwood forest. The potential economic importance of the community makes it highly desirable that its characteristics be studied and recorded.

To learn the nature of the community in which white birch is now reappearing on cutover pulpwood lands, a stand with white birch reproduction ten years of age was selected for study (summer, 1940) from a large area which had been subjected to a clean cut for pulpwood in the summer of 1930. This stand was located on Lot 11, Range 17, Byron, Oxford County, Maine. A 54–60-year-old stand, believed to be typical of those in which white birch is a dominant species in the yellow-birch subtype, was also selected for study. The sites of the two stands were comparable. The older stand was on Lot 6, Range 13, Byron, Oxford County, Maine. The origin of this 54–60-year-old stand is obscure, although it seems not to have been due to fire. Aspens were relatively unimportant in the community, and hardwoods and softwoods were of nearly the same age, both conditions atypical of a stand which immediately follows fire.

At the present time it is not possible to work out a complete birch-dominated successional series on clear-cut areas formerly occupied by the mixed spruce–hardwood type, for only occasionally on very small areas was the mixed forest cut clear prior to 1930, circa. On these restricted areas, as elsewhere, birch dominance is only local so that representative stands are commonly too small for ecological analysis. Other reasons such as obscure history or selective cutting eliminated all intermediate-aged stands observed in the Byron region. Thus it is possible now to record only the beginning of succession and to predict the trend of future changes on the basis of a mature undisturbed stand.

METHODS[2]

After the limits of each white birch community had been determined by a preliminary cruise, a series of ten sets of quadrats was laid out. Following a compass the sets were spaced at 10-meter intervals throughout the longest diameter of the community. Each set of quadrats consisted of a nest of three quadrats, the largest size being 10 by 10 meters on a side, with the smaller sizes (four by four meters, and one square meter) superimposed in one corner. On the largest plot, counts and diameters were obtained for all the woody individuals over one inch d.b.h.,[3] or over ten feet tall. Records for over- and under-story were kept separate. Shrubs and woody reproduction under one inch d.b.h., or less than 10 feet high, were counted on the four-by-four-meter plots and records were kept separately of the individuals less than one foot high and more than one foot high. Thus four woody strata were distinguished (overstory, understory, transgressives, seedlings) in the mature stand but only three in the young stand where no understory had yet developed. Herbs were listed on the one-square-meter quadrats and their coverage estimated. From these data it was possible to derive intimate knowledge of each stratum, including density and frequency of all woody plants as well as the basal area of the dominant trees in both stands and the secondary trees in the mature stand. The frequency and average coverage of the herbaceous plants were also determined.

In the young stand the stumps of the trees which formed the preceding community were in a fair state of preservation and their identity was easily determined. These stumps were counted and their distribution noted, so that it was possible to determine with a fair degree of accuracy the type of forest which previously occupied the area.

RESULTS

The nature of the stand which was clean cut for pulpwood in 1930. The nature of the community which preceded the establishment of the ten-year-old white birch community used in this study was determined by analysis of the stumps encountered on the ten-by-ten-meter plots in the young stand. The results of this study are presented in table 1.

It appears that the original stand, prior to cutting, was a mixed spruce–hardwood community with a relatively large proportion of red spruce. Of the 71 stumps encountered on the ten plots forty-eight (67.6 per cent) were red spruce and eighteen (25.4 per cent) were of the hardwood species indicated in table 1. Five balsam fir stumps were encountered, representing 7.1 per cent of the total number. White birch trees made up only 9.8 per cent of

[2] Modified after the method used by Oosting and Billings (1939).
[3] d.b.h. = diameter breast high.

TABLE 1

Frequency and density of the stumps remaining from the stand which previously occupied the area upon which a ten-year-old stand of white birch is now established. Based on ten 10 × 10-meter plots

Species	Frequency	Density
Picea rubens	100	4.8
Betula papyrifera	50	0.7
Betula lutea	30	0.6
Acer rubrum[a] and *Acer saccharum*	30	0.5
Abies balsamea	30	0.5

[a] Impossible to separate these species in the present state of preservation of the stumps.

the original stand and were irregularly distributed (frequency 50 per cent). These figures may be significant nevertheless. Factors such as seed year, germination, and early growing conditions may all serve selectively to determine what species become established on denuded forest land. But the relatively low values for white birch suggest a low minimum of parent trees

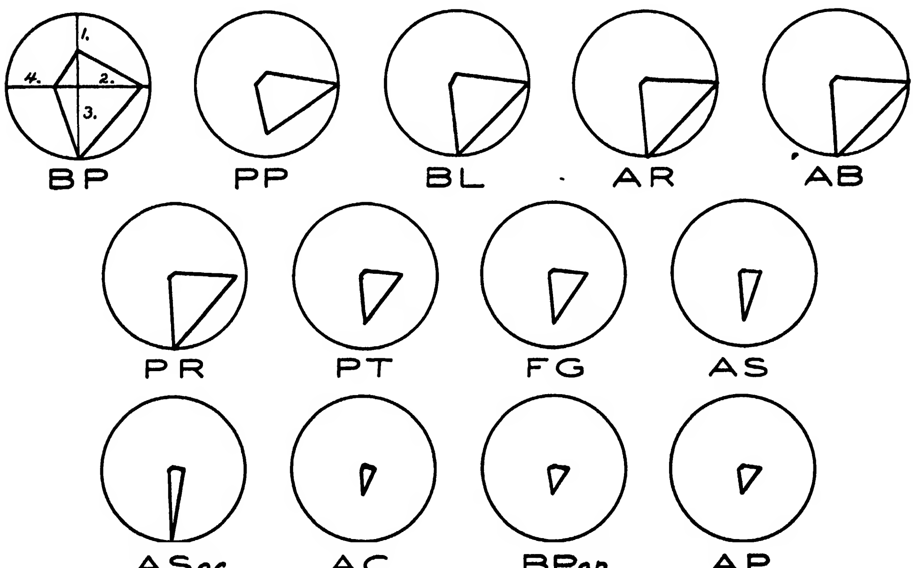

Fig. 1. Phytographs for the dominant tree species of the young white birch stand. *Radius 1.* Percentage of total dominant abundance. *Radius 2.* Percentage frequency. *Radius 3.* Percentage of total size classes represented. *Radius 4.* Percentage of total dominant basal area. In these phytographs the inner end of each radius represents the absence of its assigned sociological characteristic. All phytographs based on ten 10 × 10-meter plots. *Abbreviations:* BP—*Betula papyrifera,* PP—*Prunus pennsylvanica,* BL—*Betula lutea,* AR—*Acer rubrum,* AB—*Abies balsamea,* PR—*Picea rubens,* PT—*Populus tremuloides,* FG—*Fagus grandifolia,* AS—*Acer spicatum,* ASac—*Acer saccharum,* AC—*Amelanchier canadensis,* BPop—*Betula populifolia,* AP—*Acer pennsylvanicum.*

necessary for the establishment of a birch-dominated stand after clear cutting.

The young white birch stand in the mixed spruce–hardwood forest. The young forest which followed the clear-cut of 1930 was studied in August, 1940. At this season of the year the aestival aspect of the lesser vegetation predominated. Identification of the immature, autumnal-flowering plants was possible, but it is probable that some of the more ephemeral spring-flowering plants had withered beyond recognition.

TABLE 2

Densities and frequencies of tree species by height classes for a birch dominated stand 10 years after clear cutting of mixed spruce–hardwood and a 60-year-old undisturbed stand of unknown origin

		10-yr. stand			60-yr. stand			
		over 10 ft.	1–10 feet	under 1 ft.	over-story	under-story	1–10 feet	under 1 ft.
Betula papyrifera	d	37.8	40.0[a]	3.1	6.0	0.3	......	......
	f	90	100	20	100	20		
Prunus pennsylvanica	d	12.7	21.9	......	......	0.2	......	....
	f	100	80			20		
Betula lutea	d	10.8	13.1	3.8	0.1	0.3	5.0	45.0
	f	100	50	30	10	20	60	100
Acer rubrum	d	6.6	5.6	4.4	0.8	2.4	81.3	395.0
	f	100	60	40	50	80	90	100
Abies balsamea	d	6.0	78.1	11.3	0.5	3.2	42.5	112.5
	f	100	70	60	40	100	100	100
Picea rubens	d	3.2	25.6	1.9	1.2	3.5	2.5	6.3
	f	80	80	20	50	100	30	30
Populus tremuloides	d	2.5	1.3	......	0.4	......	4.4	2.5
	f	50	20		20		40	30
Fagus grandifolia	d	1.2	1.9	......	0.4	0.3	8.8	3.8
	f	40	30		10	30	80	30
Acer pennsylvanicum	d	1.0	......	......	0.2	......	33.8	33.1
	f	30			10		100	100
Betula populifolia	d	0.8	......	......	......	......	......	......
	f	20						
Acer spicatum	d	0.2	1.9	......	0.2	0.1	2.5	6.3
	f	20	10		10	10	20	40
Acer saccharum	d	0.2	1.9	13.6	0.2	3.1	80.0	77.5
	f	10	20	40	20	70	100	90
Amelanchier canadensis	d	0.1	......	......	0.1	1.2	......	......
	f	10			10	50		
Sorbus americana	d	......	0.6	......	......	......	......	......
	f		10					
Salix sp.	d	......	0.6	......	......	......	......	......
	f		10					
Populus grandidentata	d	......	......	......	0.5	0.1	......	......
	f				20	10		
Fraxinus americana	d	......	......	......	......	0.2	......	......
	f					10		
Prunus serotina	d	......	......	......	......	......	1.3	......
	f						10	

[a] Transgressive and seedling counts made on 4 × 4-m. plots have been raised to 10 × 10-m. basis for better comparison with numbers in larger height classes.

Dominant tree species were considered to be all those comprising the canopy (table 2). These included a miscellaneous series of thirteen species all represented by individuals from 10 to 15 feet tall. White birch, pin cherry (*Prunus pennsylvanica*), yellow birch, and aspen (*Populus tremuloides*) were consistently the tallest trees on the area, although they did not so markedly overtop the other species as to form an upper and lower division of the canopy.

White birch was clearly the most important species in the stand (fig. 1) as indicated by its density (average number of trees per quadrat) of 37.8, which is about three times that of pin cherry or of yellow birch with the next highest values. On the basis of transgressives and seedlings as well as tree counts four species other than white birch should be mentioned as important in the stand. Yellow birch, red maple, balsam fir, and red spruce all were well represented in all height classes and of these species only spruce had a frequency of less than 100 per cent in the canopy. Other species numbered among the dominants all appeared with relatively low frequencies and were all represented by density values of less than one-half the average density of all dominant species in the stand, i.e., 6.3.

The total basal area of all the dominants per 100 square meters averaged 9.81 square feet and the average per species was 0.76 square feet. Those species which had the highest densities likewise contributed the most to basal area, for all equalled or exceeded the species average: white birch, 3.76 sq. ft.; pin cherry, 1.7 sq. ft.; yellow birch, 0.79 sq. ft.; and red maple, 0.75 sq. ft.

Phytographs of the dominant species (fig. 1) serve to emphasize further the relative importance of the species at the time of sampling. The first six were obviously much more abundant, more uniformly distributed, and had a greater basal area than the last seven. In addition they were all represented in at least one more size class, which suggests that, for a time at least, they will continue to dominate the stand. Two possibilities may account for future changes in the relationships of the species with fewer size classes. If they are slow growing and shade tolerant (e.g., *Acer saccharum*), they will eventually become more and more important and predominate in all strata. If they are intolerant (e.g., *Betula populifolia*) they have already reached their peak possibilities and will soon disappear or at least not be replaced.

Pin cherry was an important competitor with white birch during the early development of this stand. The more slowly growing hardwoods, namely yellow birch, red maple, beech, and sugar maple, were definitely of less importance than birch during the first ten years of development. Spruce and balsam fir were both important species, apparently making regular and appreciable growth in competition with the surrounding hardwoods.

Twelve tree species which occupied a secondary or transgressive position

in the community had an average density of 17.3. Balsam fir, white birch, red spruce, and pin cherry were the only species represented in numbers exceeding the average for all species and with frequencies of 70 per cent or more. Thus it is clear that spruce and fir were well established in the stand. All important species of the dominant stratum were present in greater numbers in the subordinate size class except red maple. This is clearly the result of seeding over a period of several years after clear cutting, during which

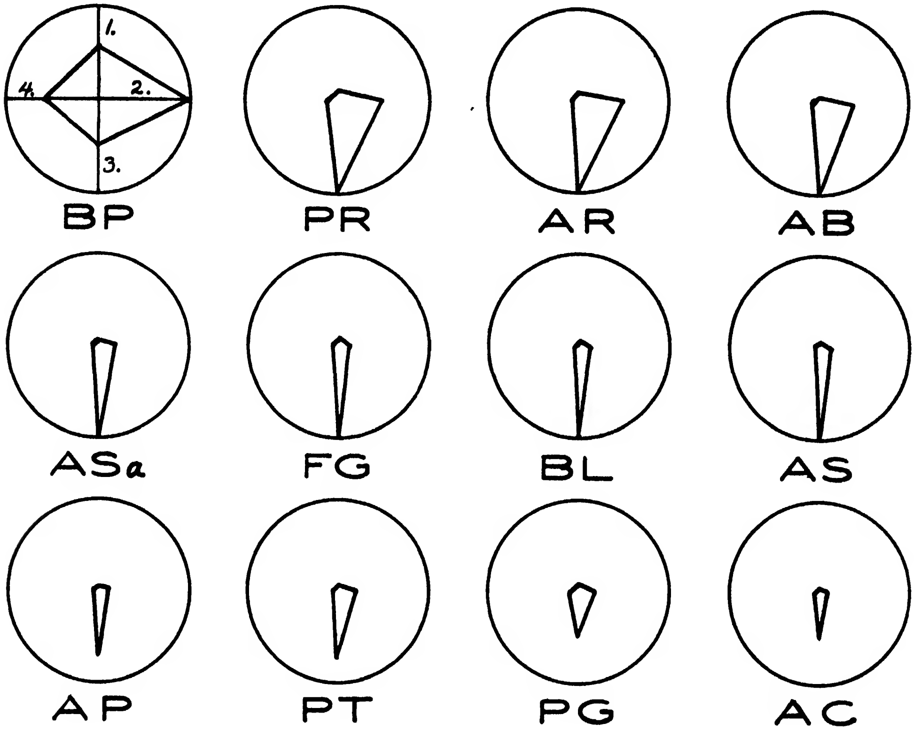

FIG. 2. Phytographs for the dominant tree species of the 54–60 year old white birch stand. Radii as in figure 1. *Abbreviations:* BP—*Betula papyrifera,* AR—*Acer rubrum,* AB—*Abies balsamea,* ASa—*Acer saccharum,* PR—*Picea rubens,* FG—*Fagus grandifolia,* AP—*Acer pennsylvanicum,* BL—*Betula lutea,* AS—*Acer spicatum,* PT—*Populus tremuloides,* AC—*Amelanchier canadensis,* PG—*Populus grandidentata.*

time a wide variety of species have almost equal opportunity to become established. After a canopy develops, as in this stand, many of these overtopped individuals should be eliminated and it would be expected that the numbers of shade intolerant species would decline rapidly. On the other hand, species which are tolerant should continue to reproduce and, therefore, form an increasingly larger percentage of the lesser size classes.

The numbers and distribution of seedlings (less than 1 ft.) become of

interest then in suggesting the changes to be expected in stand composition. Only six species were represented in this size class. Sugar maple and balsam fir made up more than one-half of the total count and were present with a relatively high frequency. Their future importance in the stand was thus assured. The low values for white and yellow birch suggest their future diminished importance in the stand and this may also be true for red spruce. Red maple values however suggest its permanency with only slight decreases in numbers.

The complete absence of seedlings of several species must be interpreted. With no reproduction to replace the short-lived dominants, pin cherry must soon disappear from the stand. Aspen is somewhat in the same category but not as certainly. Beech and mountain maple are shade-tolerant and slow-growing. Both have low mortalities, once established, and therefore an occasional good seed year is sufficient to maintain their presence in significant although never predominating numbers.

Among the shrubs (table 3) the wild raspberry (*Rubus idaeus* var. *aculeatissimus*) was so important in this young stand as to form a dense

TABLE 3

Density and frequency of shrubs in a 10-year and a 60-year birch dominated stand

	10-yr. stand		60-yr. stand	
	D	F	D	F
Rubus allegheniensis	10.6	10	0.6	10
Corylus rostrata	5.6	20	0.6	10
Ribes prostratum	3.8	20	.	.
Viburnum alnifolium	1.3	20	0.6	10
Sambucus racemosa	0.6	10	. . .	.... ..
Rubus idaeus var. *aculeatissimus*	43%[a]	90	.	. .
Cornus alternifolia	......	.... ..	5.0	20
Vaccinium pennsylvanicum	....	....	28.8	30

[a] Average estimated cover as determined on ten plots. Densities determined on 4 × 4-m. plots have been raised to 10 × 10-m. basis.

cover (43 per cent) over much of the lesser vegetation. Since its growth form is such that it sprawls over every adjacent unoccupied space, it was considered more significant to record the area covered by the species than to make a count of the individual stems on the plots. Other shrubs most characteristic of this young stand were: the beaked hazelnut (*Corylus rostrata*); the blackberry (*Rubus allegheniensis*); and the skunk currant (*Ribes prostratum*). The shrubs other than raspberry seem relatively unimportant in a stand of this age, as is indicated by the rather low value of 21.9 which represents the combined total density of all shrubs encountered per 100 square meters, raspberry excluded.

The frequency and coverage of the herbaceous vegetation under the ten-

year-old white birch stand appear in table 4. *Cornus canadensis, Aspidium spinulosum* var. *intermedium, Maianthemum canadense,* and *Viola rotundifolia* were the most widely distributed species with the greatest cover within the community. Only eleven herbaceous species were encountered on the plots, of which five were limited to a single plot. It is possible that the relatively dense shade under the abundant red raspberry cover of the shrub layer is an important factor in the explanation of this limited herbaceous population.

TABLE 4

Cover and frequency of herbs in a 10-year and 60-year birch dominated stand

	10-year		60-year	
	per cent cover	F	per cent cover	F
Cornus canadensis	20.5	60	1.5	10
Aspidium spinulosum var. *intermedium*	5.5	40	......	......
Maianthemum canadense	2.1	40	4.2	70
Viola rotundifolia	2.0	40	....	....
Osmunda claytoniana	1.5	10	. .	. . .
Carex debilis var. *rudgei*	1.1	20		.
Trientalis americana	0.7	30	2.5	40
Aralia nudicaulis	0.5	10	1.0	10
Coptis trifoliata	0.5	10	....	......
Oakesia sessilifolia	0.5	10	....	. .
Lycopodium clavatum	0.1	10	. . .	..
Pteris aquilina	......	.....	8.5	40
Lycopodium obscurum	......	....	2.0	10
Pyrola americana	....	....	0.2	20
Lycopodium complanatum	..	.	0.2	20
Fragaria virginiana	....	...	0.2	20
Gaultheria procumbens	......	.....	0.1	10
Aster infirmus	....	......	0.1	10
Chimaphila umbellata	....	. . .	0.1	10
Clintonia borealis	.....	. ...	0.1	10

The herbaceous vegetation is more nearly comparable to that of the *Cornus-Maianthemum* forest type of the Adirondacks (Heimburger 1934) than to any other type described in eastern North America. It differs from the typical *Cornus-Maianthemum* type in several of the associated species; notably, in that *Dalibarda repens, Clintonia borealis,* and *Pteris aquilina* were not found to be present here, and in that *Aspidium spinulosum* var. *intermedium* and *Viola rotundifolia* were of such importance. Perhaps this is limited information upon which to designate a forest type by comparison with those described in a similar province but the necessity for an intensive study of forest types in Maine is indicated before direct use of Cajander's (1926) forest-type method can be made.

The older white birch stand in the mixed spruce–hardwood forest. The older forest, dominated by white birch (fig. 2), was studied a few days after

completion of the field work in the young forest, already described, so the seasonal aspect of the vegetation in the two communities is comparable.

Phytographs of the dominant tree species in this stand are presented in figure 2. The white birch, 54–60 years of age, was near the peak of its marketable growth, according to the data presented by Dana (1930) for the development of merchantable volume of white birch in Maine.

The important role of white birch in this stand is indicated by the phytographs of figure 2. Its density was 6.0 as compared with 0.82, the average density for all dominants in the stand. Red spruce, density 1.2, and red maple, density 0.8, were the only other dominant species whose density values approached the average density of all dominants. The average basal area per 100 square meters of all dominants in this community was 2.52 square feet, and the total basal area of all dominants per 100 square meters was 27.74 square feet. The basal areas per 100 square meters of those species showing the greatest density values were: 17.24 square feet for white birch, 3.08 square feet for red spruce, and 1.42 square feet for red maple. Balsam fir had an average basal area per 100 square meters of 1.72 square feet and *Populus grandidentata* an average of 2.04 square feet, these being the only additional species in the stand approaching the average basal area for all dominants.

Secondary trees (table 2) included all species of the overstory except *Populus tremuloides* and only two additional species, *Prunus pennsylvanica* and *Fraxinus americana*. Individuals of a species were invariably smaller in the understory and occurred there with different densities than in the overstory. Yet their proportions in the subordinate stratum as well as their distribution were such that phytographs, constructed for ten species present in both strata, were so remarkably similar in form to those of the overstory that their reproduction is not justifiable. It is sufficient to indicate that the major species were represented in the understory by individuals with sociological values having the same relative proportions in this stratum as in the overstory. Of the important trees only white birch was an exception. Its maturity, impending decadence, and eventual elimination were strongly indicated. Its density was only 5 per cent of that in the overstory, its frequency only 20 per cent and its basal area well below the average for all species. Since all reproduction had ceased (no seedlings or transgressives, table 2) its eventual disappearance from the stand was obvious whether cut or not.

The average density of all twelve species in this stratum was 1.24. In the order of their importance the species of the secondary layer were: red spruce, density 3.5; balsam fir, density 3.2; sugar maple, density 3.1; and red maple, density 2.4. The average basal area per 100 square meters of all the secondary trees was 0.67 square feet, and the total basal area per 100 square meters of

all species was 8.06 square feet. Five species surpassed the average of all secondary trees in respect to their average basal area per 100 square meters; they were: red spruce, 4.17 square feet; balsam fir, 1.72 square feet; beech, 1.05 square feet; sugar maple, 0.79 square feet; and red maple, 0.70 square feet.

The frequency and density of the tree species of transgressive size, over one foot and less than ten feet in height, appear in table 2. Their average density was 26.19. Red maple and sugar maple were the most important hardwood species in this size class. No white birch was present. Balsam fir was much more abundant and more evenly dispersed throughout the stand than red spruce. Striped maple, a small tree of little economic or sociological value was abundantly represented.

The average density of all tree seedlings was 75.8. Seedlings of red maple, balsam fir, and sugar maple were by far the most abundant and uniformly dispersed just as in the transgressive stratum. The densities of *Betula lutea* and *Acer pennsylvanicum* were sufficiently high, however, to assure, with 100 per cent frequency, their permanence in the community if their survival continued as indicated by their values in older strata.

This community supported only a meager shrub population (table 3), both in number of species (5) and in their dispersion throughout the stand. The blueberry (*Vaccinium pennsylvanicum*) appeared most frequently and with the greatest density. It is of interest that three species (blackberry, hazelnut, hobblebush) present in the young stand were here also although in reduced numbers.

A total of thirteen herbaceous species (table 4) were encountered on the area, of which only three species were found on more than two plots. *Pteris aquilina*, *Maianthemum canadense*, and *Trientalis americana* were the most significant species on the forest floor, both as to cover and distribution. Except for the relative insignificance of *Cornus canadensis*, the herbaceous species encountered in this stand are more nearly comparable to those of the *Cornus-Maianthemum* forest type (Heimburger 1934) than to any other type described in the Adirondack region. This limited evidence again indicates that a forest-type study in the region would show markedly different vegetation complexes in the herbaceous flora beneath forest canopies than have heretofore been described in the Northeast.

INTEGRATION AND SUMMARY

This study shows that during a 10-year period, following the complete removal of a mixed spruce-hardwood forest, a mixed stand dominated by white birch and including the typical species characteristic of the mixed-spruce hardwood forest of the Northeast, has established itself. Phytosociological analysis indicates the essential similarity of the young stand and a

54–60-year-old white birch–mixed community which is the characteristic forest found on many areas on the lower slopes of the mountains in northwestern Maine.

White birch, pin cherry, yellow birch, and a limited amount of aspen together seem to be most important in establishing the first canopy on certain clear-cut sites. Abundant balsam fir, red spruce, and white birch reproduction constitute the secondary layer of the young stand when ten years of age. Dense growth of red raspberry in the shrub layer forms such a degree of shade that the very tolerant sugar maple and balsam fir are favored in the youngest size class of tree reproduction within the 10-year-old stand. The herbaceous plants of the young stand apparently constitute a community which is in part persistent from that present beneath the preceding stand, as evidenced by the importance of *Cornus canadensis* at the ten-year-old stage. This species in the region is most frequently associated with the typical mixed spruce–hardwood forest such as previously occupied the site now covered by the young stand. It does not, however, seem to persist in such an important role when the admixture of dominant species includes white birch, as is true in the 54–60-year-old stand. In part, the herbaceous layer beneath the white birch–mixed forest represents, even at an early age, certain new combinations of species destined to remain conspicuous throughout the duration of the white birch dominance. *Maianthemum canadense* and *Trientalis americana* represent such a combination in the stands studied.

Indications are that an attempt to apply the Cajander method of forest types in northwestern Maine can follow successfully only after a careful study of the communities in that specific area. The work of Heimburger (1934) in the Adirondack region does not seem to be thoroughly applicable to the forests of Maine. There is evidence from indicator species that the effect of a preceding community may be felt after cutting for at least a 10-year period, the critical period of ecesis for the new community, a fact which complicates the interpretation of herbaceous ground cover as "typical" of the forest community above it.

Because of the comparatively recent practice of clear-cutting in the mixed spruce–hardwood region of northwestern Maine, no complete successional or developmental series can be arranged for white-birch-dominated communities at this time. It is evident, however, by comparison that on those sites where white birch gains the ascendency directly after cutting, it remains to constitute the important dominant for at least 54–60 years, since no real difference exists in the complex of species in the 10-year-old community and in one selected for age alone (54–60 years) on a similar site. In the older stand the importance of white birch is obvious in that it occurred with 100 per cent frequency, and its total basal area represented 69.3 per cent of the total basal area of all the dominants. It is to be emphasized, however, that

not all of the mixed spruce–hardwood forest sites return, after clearing, to a forest in which white birch occupies such a major position. These results merely indicate that the development of a white birch–mixed forest community on any given area formerly occupied by a mixed spruce–hardwood forest is possible within 10 years after that forest has been removed. This fact has not hitherto been stressed, yet it is of importance to gauge the future supply of white birch wood in Maine for the many industries dependent upon it.

Most of the white birch in the region is cut by the time it reaches 60–70 years of age, a fact conditioned by its favorable volume, its reduced growth rate as it is overtaken in the mixed forest by the more slowly growing but longer-lived species, and its tendency to larger volumes of "red heart" (i.e., discolored heartwood) with increasing age and over-maturity. After the removal of the mature white birch, the species is not found within the stand unless future treatment provides large openings in which it may have its necessary light requirements fulfilled, or unless stump sprouts manage to survive in peculiarly favorable locations. Balsam fir, a constant associate of the white birch during its life in the mixed community, is another species which occupies a diminished importance concurrently with or slightly prior to the peak of the white birch dominance. The 60-year stand included many over-mature and dead balsam firs, but the marked reproductive capacity of the species and its tolerance of shade would seem to insure its persisting as a lesser species after the removal of the white birch.

From this study it would appear that once the white birch has been eliminated from a white birch–mixed forest community, either through senility or by selective logging, the subsequent forest remaining and persisting would be a typical mixed spruce–hardwood stand which would include red spruce, red and sugar maples, and yellow birch as its major components. It will be recalled that such a forest when clear cut may be succeeded by a young community, such as the 10-year stand, one of whose major pioneer tree species is white birch.

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BULLETIN OF THE TORREY BOTANICAL CLUB

69(9): 661–668 DECEMBER, 1942

CEPHALOMONAS, A NEW GENUS OF THE VOLVOCALES[1]

NOE HIGINBOTHAM

The members of the *Coccomonas* group, the Coccomonadae of Pascher (1927) are apparently very rare in this country since hitherto none has been reported, so far as the author has found. Therefore the discovery in Maryland of a new distinctive alga similar to the Coccomonadae in having a lorica instead of a typical cell wall seems to be of considerable interest. Since the new alga is characteristically different from both *Coccomonas* Stein and *Thoracomonas* Korschikov, the most similar genera, it appears desirable that it be given generic rank.

VEGETATIVE CELLS

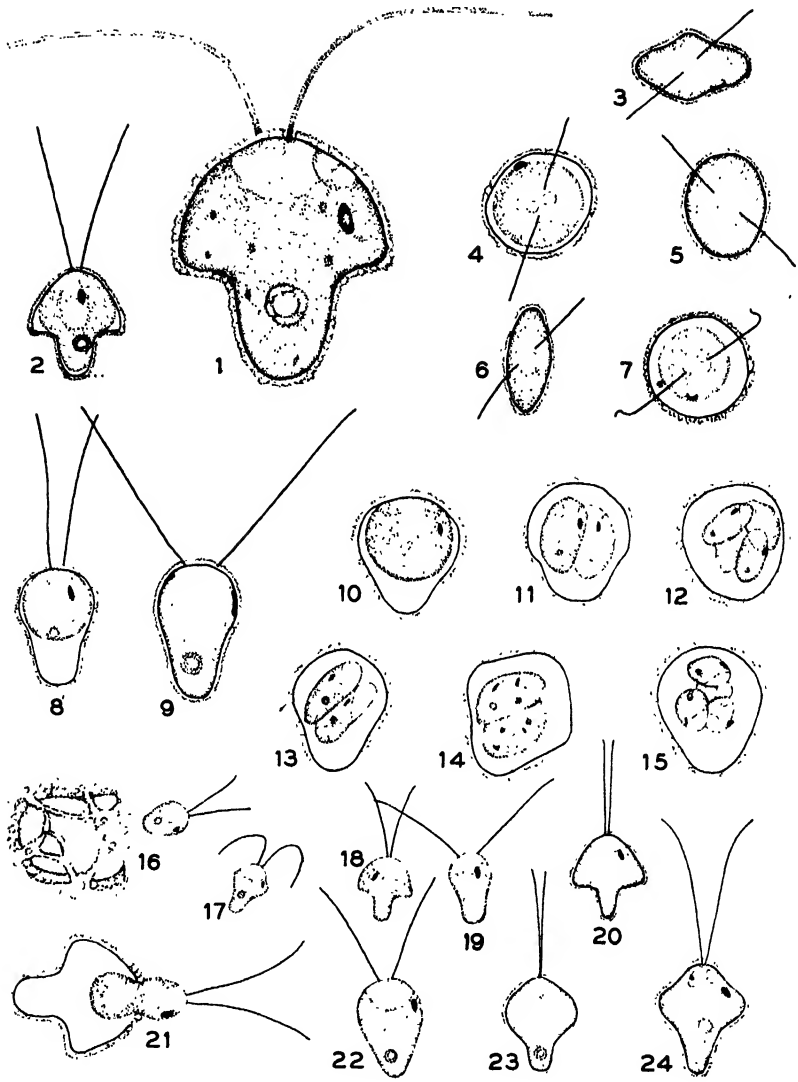
Numerous collections of this new alga were made from roadside puddles during the summer of 1941. The lorica is relatively thick, granulate, and is clear or tinged with yellow. It does not seem to turn brown with age as do the ferric-impregnated shells of other algae such as *Trachelomonas* and *Thoracomonas*. It seems rigid in older cells, however, since it breaks when pressure is applied on the cover glass. The protoplast usually fills the lorica (fig. 1) although occasional individuals are found in which the naked protoplast has contracted and rounded up inside the shell (figs. 2, 4, 8). This condition also occurs prior to reproduction (figs. 7, 10). The two anterior flagellar pores are relatively widely separated (fig. 1).

The form of the cells (figs. 1, 2) may best be compared to that of a truncated *Brachiomonas simplex* individual. There is an anterior, subhemispherical, head-like portion which ends abruptly at about the middle, and a cylindrical, slightly tapered, posterior process. The cells are always somewhat compressed and appear pyriform in side view. In optical transverse section the cells are elliptic, or obscurely rhomboidal (figs. 3–6). Not infrequently the compressed sides project slightly (fig. 3). Older cells preparing to divide become rounded (figs. 4, 7). The cells range from 8 to 15 μ long, from 6 to 12 μ wide (face view), and from 5 to 9 μ thick. The flagella are from 8 to 16 μ long, being equal to or greater than the body length.

The insertion of the flagella is oblique with reference to the transverse axis (figs. 3–6) as in the Phacotaceae.

The protoplast has the usual *Chlamydomonas*-like structure. The

[1] Contribution from the Chesapeake Biological Laboratory and from the Osborn Botanical Laboratory of Yale University with the aid of the Theresa Seessel Research Fellowship for 1941–42. The author is very grateful to Professor Tracy E. Hazen and to Professor Harold C. Bold for criticism of the manuscript.



FIGS. 1–24. *Cephalomonas granulata.* FIG. 1 × 2720. FIGS. 2–24 × 1370. FIG. 1. Vegetative cell in face view. Two contractile vacuoles near base of flagella, pyrenoid in posterior process, small granules scattered in cytoplasm. FIG. 2. Cell in face view, protoplast contracted from lorica. FIGS. 3–7. Cells in optical section (seen from above). Figures 3 and 6 show more typical vegetative cells; cells in figures 4, 5, and 7 probably preparing for reproduction. FIG. 8. Side view of a contracted cell. FIG. 9. Side view of ordinary cell. FIGS. 10–15. Stages in zoospore formation. FIG. 10. Protoplast in preparation for first division. FIG. 11. Stage following first division. FIG. 12. Stage following second division. FIG. 13. Stage following first division. FIG. 14. Second division in process. FIG. 15. Four zoospores. FIG. 16. Disintegration of lorica and escape of naked zoospores. FIG. 17. Face view of zoospore recently liberated. FIG. 18. Face view of young cell, still naked and slightly metabolic. FIG. 19. Side view of young cell. FIG. 20. Young cell with

chromatophore is large and cup-shaped, filling the cell except for an anterior, more or less axial, clear region (figs. 1, 9). It is a light grass-green in the thinner cells but the larger individuals are darker in color. There is one small pyrenoid body (sometimes lacking) usually located in an axial position in the posterior process (fig. 1). The only starch found in the cell occurs as a layer around the pyrenoid. Other granules are present scattered throughout the cell and these may represent a reserve carbohydrate which does not react in the iodine test. No fatty substances have been demonstrated by the usual methods. The red eye-spot is slightly elongated, oval or elliptical in form, and is located laterally near the anterior third of the cell (figs. 1, 3). There are two contractile vacuoles in the clear region at the anterior end (figs. 1, 4, 6).

A most unusual feature of the cell is the lateral, not axial, position of the nucleus (figs. 36, 39, 41). The studies of the nucleus were made on material fixed in Carnoy's solution and it is possible that this appearance is an artifact. The prepared slides, however, showed that the nucleus often occupies a position on one side of the cell next to the shell. As may be seen in figures 36 and 37 the nucleus has a visible chromatin content.

The cells are extremely active, swimming with a spiral movement and capable of reversing at any time. They seem remarkably sensitive to light, as compared to *Pandorina*, *Chlamydomonas*, *Phacotus*, and other organisms. When a mixture of the above forms and the new alga was placed in a drop of water on a slide, nearly all the *Cephalomonas* cells gathered on the more intensely lighted side within a minute, whereas, under the same conditions, the other organisms reacted little or not at all in that length of time.

ASEXUAL REPRODUCTION

Asexual reproduction takes place with the formation of 2–4 motile naked zoospores. Nearly all cell division stages were observed in the evening or early morning. When the cells are preparing to divide they tend to round up. The protoplast then shrinks away from the shell and the organism soon comes to rest, losing its flagella (fig. 10). No cell division has been observed in motile cells. The first division is longitudinal with respect to the protoplast although the latter may change its position somewhat within the shell. Sometimes only two daughter cells are produced but there is usually a second division forming four daughter cells (figs. 12, 15). So far as has been ascertained the second division is also parallel to the long axis of the protoplast. Following the second division the four daughter cells, or zoospores, round up somewhat, and then gradually assume a pyriform shape.

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newly-formed, relatively smooth shell. Fig. 21. Escape of whole protoplast, from lorica. Fig. 22. Protoplast of figure 21 immediately after its escape. Figs. 23, 24. Young vegetative cells.

In the meantime the lorica of the parent cell has swollen considerably and the granules begin to separate from it. As the zoospores become active with the appearance of their flagella the lorica disintegrates irregularly into small granules and fragments of various size (fig. 16), thus liberating the young, naked, somewhat metabolic, cells (figs. 16, 17). The biflagellate zoospores are 4–6 μ long and soon assume the shape (fig. 18) of the parent cell. The shell appears first as a thin smooth layer closely investing the cell and then gradually becomes granulate with age. Cells in hanging drop culture sometimes retained a smooth non-granulate lorica, possibly because of the absence of iron.

An entire protoplast was once observed escaping from its lorica (fig. 21). This cell assumed a pyriform shape (fig. 22) like that of a zoospore, but eventually resumed its original form.

The zoospores are essentially miniatures of the parent cells as to chromatophores, eye spot, etc. The pyrenoid was often not visible in zoospores observed in the morning in hanging drop cultures but later in the day all cells seemed to have one. Professor Hazen has observed the same phenomenon in a species of *Chlamydomonas*.[2]

SEXUAL REPRODUCTION

In sexual reproduction two biflagellate naked gametes, alike in size or nearly so, fuse to form a spherical quadriflagellate zygote. Sexual reproduction has been observed on two separate occasions between 6 a.m. and 9 a.m., with the formation of many zygotes in each case. The gametes are produced in the same manner as the zoospores, from which they cannot be distinguished morphologically with certainty. As in zoospore formation the divisions of the parent cell to form gametes were never observed to produce more than four cells, but the slightly smaller average size of the free gametes indicates that perhaps eight cells may often be formed. The gametes are pyriform, slightly metabolic, and range from 3–5 μ in length. Each cell has a chromatophore and a stigma but the pyrenoid appeared to be lacking more often in the gametes than in the zoospores. In the absence of structural differences between zoospores and gametes the only reliable criterion for the recognition of gametes is their capacity for sexual fusion.

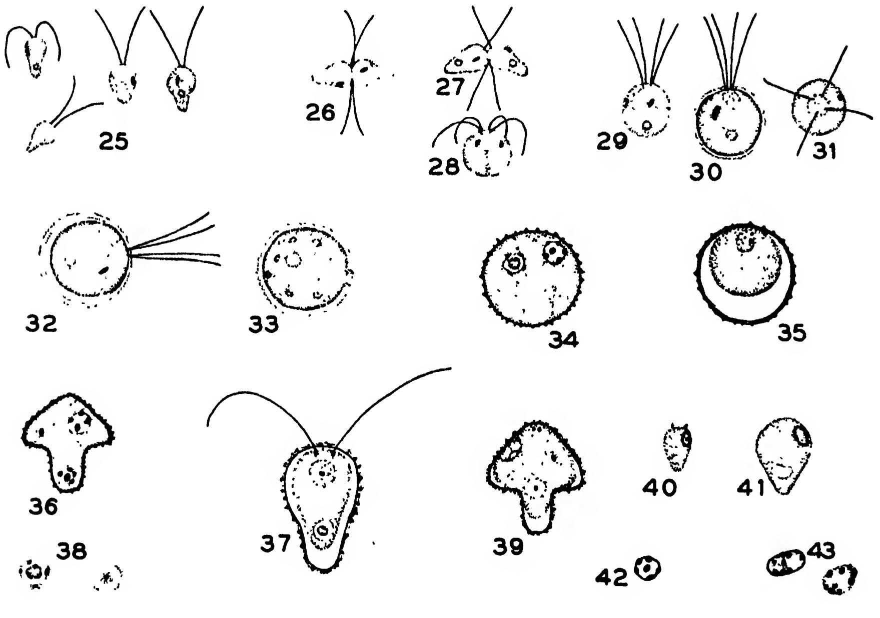
In the sexual fusions that were observed, two equal, actively motile gametes became attached at their anterior ends (figs. 26, 27). During the fusion process they swam about very actively, making it difficult to observe details. The newly formed zygotes are spherical and actively motile. Each contains two stigmas and two chromatophores at first (fig. 28). Later the chromatophores fuse (fig. 29) and eventually only one eye spot is visible (figs. 30, 31). The zygotes remained active (in hanging drop cultures) for

[2] Personal communication.

1–3 days and during this time they formed a granulate shell or spore wall (fig. 30) and increased in size from 6–8 μ to 8–12 μ in diameter. Following this vegetative phase they came to rest (fig. 32) and eventually lost their flagella (fig. 33). The protoplast not infrequently fails to fill the shell lumen (figs. 32, 35). Zygospore germination has not been observed.

CYTOLOGY

Fixed and stained individuals have supplied a few cytological details. With iron-alum hematoxylon the granules in the lorica take a deep stain but no distinct layers can be discerned in the shell matrix (figs. 36, 37, 39). A darkly staining granule, the blepharoplast (figs. 37, 39), lies at the base of each flagellum. A rhizoplast was not observed. In most cells the cytoplasm contains a number of small vacuoles (figs. 36, 39), but these are not so



FIGS. 25–43. *Cephalomonas granulata.* All figures × 1370. FIG. 25. Isogametes. FIGS. 26, 27. Attachment of gametes to one another. FIG. 28. Newly formed zygote still with two eye spots and with a faint line between the chromatophores. FIG. 29. Same with chromatophores fused and one pyrenoid apparent. FIG. 30. Motile zygote with newly formed wall. FIG. 31. Optical section of naked zygote showing insertion of flagella. FIG. 32. Zygote with protoplast contracted. FIG. 33. Zygospore. FIGS. 34–43. Fixed and stained material. FIG. 34. Zygospore showing pyrenoid (left) and nucleus (right). FIG. 35. Older zygospore, nucleus in upper portion. FIG. 36. Young vegetative cell. The pyrenoid in posterior process, nucleus, with its chromatic granules, in anterior right. FIG. 37. Side view of vegetative cell showing blepharoplasts at base of flagella. FIG. 38. Pyrenoids. FIG. 39. Vegetative cell with two blepharoplasts anterior, nucleus to left by shell. FIG. 40. Zoospore. FIG. 41. A naked (young?) protoplast. FIG. 42. Nucleus of vegetative cell. FIG. 43. Nuclei of zygotes.

conspicuous in individuals with protoplasts contracted away from the lorica (fig. 37). The pyrenoid sometimes appears simply as a clear region (figs. 40, 41), but usually a more or less darkly staining body can be seen at the center (figs. 34, 36, 37, 38, 39). Not infrequently overstained individuals show a layer of starch around the pyrenoid (figs. 36, 38).

The nuclei of vegetative cells have finely divided chromatin which rather obscurely appears to be in the form of a net (figs. 37, 39). It cannot be stated with certainty whether or not a nucleolus is present. In some cells a single nucleolus-like body occurs (fig. 37), but in other cells no such body was seen (figs. 39, 41). Not infrequently the nuclei are characterized by having a number of chromatic granules (figs. 36, 42) such as those described in *Carteria* by Akins (1941). So far as could be ascertained the number of these granules is not constant but ranges from about 4 to 7 in the vegetative cells (see figs. 36, 42), while some of the zygote nuclei have as many as 10 (see fig. 43). In addition to the chromatic granules, other chromatin material is usually discernible (fig. 43). Stages in cell division and gamete fusion were not found in the prepared slides. The appearance of the zygotes may be seen in figures 34 and 35.

LORICA

The lorica of this new alga, and of the similar genera *Coccomonas, Pedinopera,* and *Fortiella,* is a distinctive feature of a number of the unicellular Volvocales. As yet, however, little is known of the basic structure of the lorica nor of the significance of its impregnation with compounds of calcium or iron. Conrad (1930) has described *Coccomonas* as having a cellulose cell wall, in addition to the lorica, but this structure was not found in *Cephalomonas.*

A number of tests were made on the lorica of *Cephalomonas.* With potassium ferrocyanide and hydrochloric acid a blue color results, indicating the presence of the ferric ion. Since normally the lorica is not brown but clear or yellowish, it seems likely that iron is not present as an oxide, but may be combined with a shell constituent. The tests for calcium showed that none is present. The usual tests for cellulose and hemicellulose have also been applied and have failed to indicate the presence of either. These include the chloriodide of zinc color reaction, the sulfuric acid and iodine color reaction and hydrolysis with 3 per cent hydrochloric acid. Owing to the small size of the cells and the inadequate number of them available for study, these tests cannot be considered conclusive, but they are reported here for any possible value they may have for future work. It should also be remembered that some of the hemicelluloses do not hydrolyse readily. Finally, staining with ruthenium red has revealed no pectic compounds.

DISCUSSION

In having a lorica impregnated with an iron compound, *Cephalomonas*

seems to be allied with the *Coccomonas* group of the unicellular Volvocales. Of this group both *Pedinopera* (Pascher 1927) and *Fortiella* (Maggs 1941) may have iron-impregnated shells, although some species, as in *Coccomonas*, may have calcified loricas. Presumably calcium and iron both may be present in the shell of some forms (Printz 1927).

Cephalomonas is also very similar to *Thoracomonas* and it would seem that the latter genus, with its lorica, stands closer to the Coccomonadae or to the Phacotaceae (Smith 1933) rather than being close to *Chlamydomonas* as Printz (1927) suggests.

The cell envelopes in the Phacotaceae are also incrusted with calcium or iron, or both, and Fritsch (1935) includes *Coccomonas* in that family. Pascher (1927), on the other hand, separates the *Coccomonas* group as a tribe, the Coccomonadineae, and limits the Phacoteae to forms with shells showing two distinct halves. The classification seems to depend chiefly on whether the lorica or the number of flagella is considered to be the fundamental feature. Printz (1927) places the biflagellate *Coccomonas* in the Phacoteae and the quadriflagellate genera, *Pedinopera* and *Fortiella*, in the Carterioideae. Such a classification, however, indicates that the lorica is essentially homologous with the wall of *Carteria* and its allies, whereas the lorica may really represent a distinctly separate development.

As has been remarked before, the forms with a lorica consist essentially of a naked protoplast within an outer capsule. Thus forms such as *Cephalomonas, Fortiella,* and *Pedinopera* may be most closely allied to the primitive naked Volvocales, the Polyblepharidaceae, and may represent an intermediate group leading to the Phacotaceae. There is considerable evidence that the Phacotaceae are a natural group climaxing a separate line of evolution, and most authors, on the basis of the presence of the cell wall, seem to believe this line arose from a *Chlamydomonas* type. It remains to be seen, however, whether or not the wall of the *Chlamydomonas*-type cell is actually homologous with the wall or lorica of the Phacotaceae and the lorica of other forms. Also it may be found that not all shelled forms are related to one another. At the present it remains a distinct possibility that the genera having a lorica may have had an independent origin from primitive volvocalian stock.

DIAGNOSIS

Cephalomonas Higinbotham, gen. nov. Cellulae vegetativae in lorica rigida ferro-crustata conclusae, semper compressae, a facie visae partem semiglobosam et processum posteriorem conspicuum habentes; a latere pyriformes; a sectione transversa ellipticae vel obscure quadrangulares. Flagella duo, anteriora, aequalia, e poris separatim per loricam exeuntia. Stigma praesens. Pyrenoides pro more praesens, interdum absens. Amylum circum pyrenoiden in strato dispositum. Vacuola contractilia duo, anteriora. Chromatophorum gramineo-viride, parietale, urceolatum. Propagatio per zoosporas

gametaque nuda mobilia ut videtur non structura distinguenda, loricae per dissolutione in fragmenta minuta liberata.

TYPUS: *C. granulata.*

Cephalomonas granulata Higinbotham, sp. nov. Lorica granulata, pellucida vel lutea. Cellulae vegetativae maturae 8–15 μ longae, 6–12 μ latae (a facie), 4–8 μ crassae. Flagella corpus aequantia vel paullo excedentia. Stigma ellipticum in parte tertia anteriore cellulae dispositum. Chromatophorum continuum, urceolatum. Pyrenoides axilis, pro more in processu posteriore, interdum absens. Nucleus anterior, pro more lateralis. Propagatio per 2–4 zoosporas nudas 4–6 μ longas. Generatio sexualis per isogametorum conjunctionem 3–6 μ longorum nudorum. Zygotum quadriflagellatum, plures horas mobile, demum membranam crassam efformans. Zygospora globosa 8–12 μ diametro immobilis.

TYPUS in stagnis parvis prope Solomons, Calvert County, Maryland, lectus; in herbario Horti Botanici Noveboracensis depositus.

SUMMARY

Cephalomonas granulata gen. et sp. nov. is described and illustrated. The new alga is unicellular and has a granulate, iron-impregnated lorica with two anterior pores through which the flagella emerge. It thus seems to be allied to the genera comprising the Coccomonadae of Pascher's classification (Pascher 1927).

Asexual and sexual reproduction take place with the formation of naked zoospores and zoogametes. The disintegration of the lorica into small fragments frees the reproductive cells.

The structure of the lorica is discussed and some cytological details of the protoplast are described. An unusual feature is the generally lateral position of the nucleus.

It is suggested that the group of organisms having loricas instead of typical cell walls may represent a separate evolutionary line which originated from the primitive naked forms of the Volvocales.

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THE INTERACTION BETWEEN THIAMINE AND FOUR FUNGI

FREDERICK KAVANAGH*

HISTORICAL REVIEW

"Vitamine" was the name created by C. Funk (4) for a growth substance needed in small amounts to prevent certain diseases; the name signified that crystals which he prepared from rice polishings behaved like an amine and prevented death of pigeons with polyneuritis and of men with beri-beri. Most of these "vitamine" preparations, which came to be called vitamin B, contained many substances,[1] some of which increased the growth of fungi in experiments of Bachmann (1), Williams (26), and Willaman (25). Lepeschkin in 1924 reported work he had done in 1916 in which a highly purified preparation of vitamin B was used. He observed that it stimulated fermentation and growth of one strain of yeast and growth of *Penicillium glaucum.*

During the decade ending in 1934, the original vitamin B preparations were discovered to contain several vitamins of which the antineuritic vitamin (vitamin B_1) had the most spectacular action on animals. Some of the investigators who were interested in the effects of vitamins on the growth of fungi used vitamin B_1 concentrates; but before much interest could be aroused in the relation between vitamin B_1 and the growth of fungi, it was necessary to synthesize the vitamin from compounds of known structure and no vitamin-like effect on animals. The modern work with vitamin B_1 started after Windaus and his co-workers (29) had prepared the crystalline natural vitamin in sufficient quantity to permit extensive experimentation and after Burgeff (3) had demonstrated that it was necessary for the growth of *Phycomyces nitens* and *P. blakesleeanus*. Schopfer (19), who was working with the effect of vitamin B concentrates on *Phycomyces blakesleeanus*, obtained striking improvement in growth with vitamin B_1, and became interested in its general importance as a growth substance for fungi.

Crystalline vitamin B_1 became available here in 1934 as a result of the work of R. R. Williams and his associates (28). In 1936 Williams and Cline synthesized the vitamin from its two intermediates, 2-methyl-5-bromomethyl-6-amino pyrimidine and 4-methyl-5-β-hydroxyethyl-thiazole.[2] Robbins and

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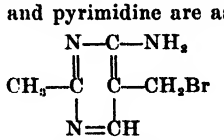
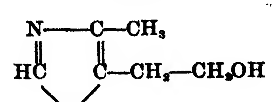
[1] The vitamin B complex consists of at least 12 members, of which 7 have been synthesized and one other has been prepared in crystalline form.

[2] The structures of the two intermediates, which will be designated hereafter simply as thiazole and pyrimidine are as follows:



pyrimidine

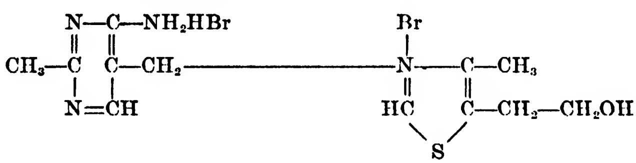


thiazole

Bartley (12, 13) showed that either vitamin B_1 or the thiazole intermediate was essential for unlimited growth of excised tomato roots. This result suggested the possibility that *Phycomyces blakesleeanus* did not respond specifically to vitamin B_1 but to one or both intermediates. Independently and simultaneously Schopfer and Jung (21) and Robbins and Kavanagh (14) demonstrated that the two intermediates together could be substituted for vitamin B_1 in determining the growth of *Phycomyces blakesleeanus*. Robbins and Kavanagh reported that molecularly equal quantities of the two intermediates taken together allowed the same extent of growth as the molecularly equivalent amount of vitamin B_1.

Robbins (11), Robbins and Kavanagh (15), Leonian and Lilly (6), Schopfer (20), and others made surveys of the vitamin B_1 requirements of a large number of fungi and found some that required vitamin B_1, some that required both intermediates, others that would grow if given only one intermediate, and still others that needed no external supply of vitamin B_1 or its intermediates. However, it seems that all living organisms require a continuous supply of vitamin B_1. The organisms which grow in a medium lacking vitamin B_1 synthesize it from the constituents of the medium (18).

The structure of the bromide-hydrobromide of vitamin B_1, as determined by the synthesis of Williams and Cline (27), is as follows:



Williams gave vitamin B_1 the name "thiamine" to indicate that it is a sulphur-containing amine. This name is preferable to "aneurin," used by European writers, a name based on its supposed function for mammals. The name "thiamine" is accepted by the American Chemical Society and is used generally in the United States. The synthetic commercial thiamine used in this paper is the chloride-hydrochloride and contains about four per cent of hygroscopic water.

THE PROBLEM

Interest has centered for several years on the effect of thiamine and related compounds on the growth of many species of fungi and on the behavior of the fungi when the thiamine requirement is or is not fulfilled. The closely related problem of what the fungi do to the thiamine has been studied only by Bonner and Buchman (2), who reported that *Phycomyces blakesleeanus* destroyed thiamine by liberating the pyrimidine and decomposing the thiazole, or, at least, altering the thiazole until it was ineffective. They presented evidence that thiazole was destroyed only when it was part of a thiamine molecule. When thiazole and pyrimidine were present in the same culture

solution, the actively-growing organism combined them rapidly to form thiamine, then destroyed the thiamine, freeing the pyrimidine from which thiamine could be formed again until all the thiazole was used. A pyrimidine molecule would thus be used several times when more thiazole than pyrimidine was supplied. They found also that *P. blakesleeanus,* when grown in a solution containing more thiazole (free and combined) than pyrimidine, produced heavier mycelia than when it was grown in a solution in which the thiazole and pyrimidine were present in equivalent amounts. This observation was contrary to that reported by Robbins and Kavanagh (14), but was confirmed later (17).

The present investigation was designed to determine (1) if pyrimidine accumulates and thiazole disappears from a thiamine solution in which *P. blakesleeanus* grows, (2) how *Phycomyces* affects free thiazole and free pyrimidine, (3) how other fungi with different requirements affect thiamine, thiazole, and pyrimidine.

MATERIALS AND METHODS

To study these problems four organisms with different needs for thiamine or its intermediates were selected: *Phytophthora cinnamomi* (15) which requires thiamine as such, *Phycomyces blakesleeanus* (14, 20, 21) which uses the two intermediates (together) as satisfactorily as it does thiamine, *Mucor ramannianus* (9) which grows with either thiazole or thiamine, and *Sclerotium rolfsii* (15) which grows with either pyrimidine or thiamine. All these fungi develop well in synthetic solutions containing thiamine or the intermediate or intermediates functional for each. *Phytophthora* and *Phycomyces* were used in the studies of Bonner and Buchman; *Mucor ramannianus* is the only fungus known to require only the thiazole portion of the thiamine molecule; and *Sclerotium rolfsii* grows well in nutrient solutions if given only the pyrimidine portion of the thiamine molecule.

The sources of the cultures were as follows: *Phytophthora cinnamomi* Rands from C. M. Tucker; *Phycomyces blakesleeanus* Burgeff from A. F. Blakeslee (this is the plus strain used in the earlier experiments of Robbins

TABLE 1. *Basal solutions*

	A	E	F
KH_2PO_4	15 g.	1.5 g.	1.5 g.
$MgSO_4 \cdot 7H_2O$	5 g.	0.5 g.	0.5 g.
Mineral supplements[a]	1 ml.	0.5 ml.	0.5 ml.
Asparagine	10 g.	10 g.	10 g.
C.P. dextrose	100 g.	100 g.	100 g.
Water to make	1000 ml.	1000 ml.	2000 ml.

[a] H_3BO_3, 5.7 mg.; $CuSO_4 \cdot 5H_2O$, 18.6 mg.; ammonium molybdate 85 per cent, 3.6 mg.; gallium sulfate, 6.8 mg.; $FeNH_4(SO_4)_2 \cdot 12H_2O$, 173 mg.; $MnSO_4 \cdot 4H_2O$, 8.1 mg.; $ZnSO_4 \cdot 7H_2O$, 79 mg.; water to make 100 ml. This is a modification of a mineral mixture used by Steinberg (22).

and Kavanagh); *Mucor ramannianus* Möller from the Centraalbureau voor Schimmelcultures at Baarn; and *Sclerotium rolfsii* Sacc. from C. M. Tucker.

The basal solutions used for the investigation of the metabolism by the four fungi of thiamine and its intermediates were composed of mineral salts, sugar, and asparagine. Their composition is given in table 1.

Although the four fungi grow quite well in solutions supplied with potassium dihydrogen phosphate and magnesium sulfate of the usual "Analytical Reagent" grade as the only mineral salts, additional micro-elements were supplied. The water was once distilled from an electric Stokes still with block tin condenser. The sugar was "C.P. Dextrose" from the Corn Products Company. The asparagine was purified by recrystallization three times from water and/or aqueous alcohol.

Three lots of each basal solutions were prepared for the study of the metabolism of thiamine and its intermediates; one lot received thiamine only; a second received thiamine and thiazole; and a third lot received thiamine and pyrimidine (table 2). By the use of these three types of solutions, it was hoped to show the effect of these fungi on thiamine and its intermediates as well as the effect of large amounts of free thiazole and free pyrimidine on the growth of the four fungi. To solution F, used only with *P. cinnamonii*, large amounts of thiamine were added in place of the large amounts of thiazole or pyrimidine used in the experiments with the other fungi. The amounts of thiamine, pyrimidine, or thiazole used in the basal solutions given in table 1, together with the volumes of solution per flask, are shown in table 2.

In previous experiments Robbins and Kavanagh have found that 1 mμmole[3] of thiamine per flask gives good growth with all these fungi and that this amount of thiamine seems to be the limiting factor for their growth in these solutions. In order to be sure that thiazole or pyrimidine was present in large excess, 115 mμmoles[4] of thiazole or pyrimidine were added to each flask containing thiazole or pyrimidine. The vitamin composition of the nutrient solutions is shown in table 2.

The procedure used in growing the four fungi in the presence of thiamine

[3] The amount of thiamine, thiazole, and pyrimidine is expressed in terms of millimicromoles (mμmoles, 10^{-9} gram-moles) per culture flask. In some previous papers the millimicromole was referred to as a unit (u.) of thiamine. It seems preferable to omit "unit" from the terminology because of the possibility of confusing it with the other four units that have been used to express amounts of vitamin B_1. Thiamine and its intermediates are expressed in amounts per culture rather than in amounts per volume of liquid, for example per liter, because usually it is the total quantity of thiamine, thiazole, or pyrimidine in a solution and not its concentration that is important.

[4] The absolute values for thiamine, thiazole, and pyrimidine may be in error by 10 per cent because of uncertainty of the water content of the compounds. The relative values given in the table are based on determination of the thiazole and pyrimidine by the *Phycomyces* method, using thiamine as the standard.

TABLE 2

Nutrient solution	A 1	A 2	A 3	E 1	E 2	E 3	F 1	F 2	F 3
Thiamine, mμmoles/flask	1	1	1	1	1	1	1	10	100
Thiazole, " "		115			115				
Pyrimidine, " "			115			115			
ml. solution used per flask	25	25	25	25	25	25	50	50	50

or its intermediates was as follows: 25 ml. portions of the nutrient solutions (table 2) were placed in 125 ml. Erlenmeyer flasks, plugged with cotton-wool, and autoclaved at 110–112° C for fifteen minutes. *Phycomyces* and *Mucor* cultures were inoculated with spore suspensions; *Phytophthora* and *Sclerotium*, with mycelium. The *Phycomyces* inoculum was prepared by placing several mature sporangia in from 10 to 25 ml. of sterile distilled water. The *Mucor* inoculum was prepared by rubbing the surface of a culture containing mature spores under a little water and adding this water to from 10 to 25 ml. of sterile distilled water. One drop of the spore suspension was added to each flask from a sterile pipette. Bits of mycelium from *Phytophthora* or *Sclerotium* stock cultures were transferred to a thiamine-free solution and allowed to grow from 5 to 7 days to form a nearly vitamin-free inoculum for the nutrient solutions. Small bits of the nearly vitamin-free mycelium were transferred to the culture flasks.

The flasks, after inoculation with the appropriate fungus, were placed on one shelf of a darkened incubator which maintained the temperature of the solutions between 24° and 25° C. The fungi were grown the desired length of time, removed from the flasks, washed with distilled water to remove salts and sugar, and dried in aluminum pans at 105° C for at least twelve hours. The dried mycelium was allowed to stand at room temperature until it reached equilibrium with atmospheric moisture,[5] and was weighed to the nearest milligram. The medium and wash water were combined and diluted to a standard volume, aliquots of which were analyzed for thiazole and pyrimidine. Single cultures were analyzed in the A series, duplicate cultures in the E series, and quadruplicate cultures in the F series.

The amounts of thiazole and pyrimidine in the solutions were so small that a biological method of assay was necessary; no chemical method of sufficient sensitivity was available. *Phycomyces* seemed to be the best of several fungi that could be used to determine thiazole and pyrimidine.

The method of assay for thiazole was as follows: A series of flasks containing an analyzing solution[6] and known amounts of thiazole (from 0.125

[5] The oven-dried mycelium after attaining equilibrium with an atmosphere of 50 per cent humidity increased about 3 per cent in weight.

[6] The analyzing solution was made with the following amounts of nutrients per flask: KH_2PO_4 37.5 mg., $MgSO_4 \cdot 7H_2O$ 12.5 mg., mineral supplements 0.0125 ml., ammonium glutamate 200 mg., dextrose 2500 mg., thiazole (or pyrimidine) 115 mμmoles. Ammonium

to 2 mμmoles per flask) with a considerable excess of pyrimidine (115 mμmoles per flask) was prepared in quadruplicate, sterilized and inoculated with *Phycomyces blakesleeanus*. The inoculated flasks were placed in a dark incubator at 25° C. The dry weight of the mycelium was determined after from 9 to 15 days of growth. A standard curve was constructed by plotting on log-log coordinate paper the average dry weight of a mycelium as ordinate and the amount of thiazole which was put into the flask as abscissa (fig. 13). This curve showed the dry weight produced by *Phycomyces* with different and known amounts of thiazole in the presence of an excess of pyrimidine under the conditions of the experiment. At the same time, aliquots of the solutions to be analyzed were added to the analyzing solution to which the same excess of pyrimidine (115 mμmoles per flask) that was used in preparing the standard curve had been added. Duplicate aliquots of each solution were used in each analysis for thiazole. The aliquots were selected to represent either half the contents of one flask or between 0.5 and 2 mμmoles of the intermediate being determined. In the E series of solutions, the contents of two flasks were combined and diluted to 100 ml. The largest aliquot taken was 25 ml., which represented one-half the contents of one flask. In the E 1 series of solutions, two 25 ml. aliquots were used for determining thiazole and two 25 ml. aliquots were used for determining pyrimidine. In the E 2 series, two 25 ml. aliquots were used for determining pyrimidine and several pairs of aliquots which ranged from 0.5 ml. to 10 ml. for determining thiazole. In the E 3 series, two 25 ml. aliquots were used for determining thiazole and several pairs of aliquots which ranged from 0.5 to 10 ml. for determining pyrimidine.

The cultures were inoculated with *P. blakesleeanus*. From the dry weights obtained the quantity of thiazole in the aliquot was read from the standard curve (fig. 13). This quantity was the total functional thiazole (free thiazole as well as thiazole in thiamine, cocarboxylase, and other forms) present in the aliquot. A similar procedure was followed in assaying for total pyrimidine except that the standard curve was constructed from dry weights obtained with known amounts of pyrimidine in the presence of a large excess

glutamate was used as the nitrogen source in place of asparagine, the supply of which was temporarily limited. The growth of *Phycomyces* in the analyzing solution was not limited by the amounts of salts, sugar or nitrogen. This solution could give growth of *Phycomyces* twice as large as any obtained in the assays. The sugar, minerals and asparagine added in the aliquots were considered not to influence the growth of *Phycomyces* in the analyzing solutions. In these analyses an aliquot was selected such that the growth of *Phycomyces* was limited by the amount of thiazole or the pyrimidine in the aliquot to less than one-third that which could be obtained in the presence of a large amount of thiamine. After the aliquots of the solutions in which the four fungi grew and the analyzing solutions were put into the flasks, distilled water was added, where necessary, to make the volume of liquid the same in all flasks (including the standards). Total volumes of 35, 40, and 50 ml. (in 125-ml. Erlenmeyer flasks) were used.

of thiazole (115 mμmoles per flask) and the aliquots of the solution to be analyzed were added to the analyzing solution containing a large excess of thiazole (115 mμmoles per flask).

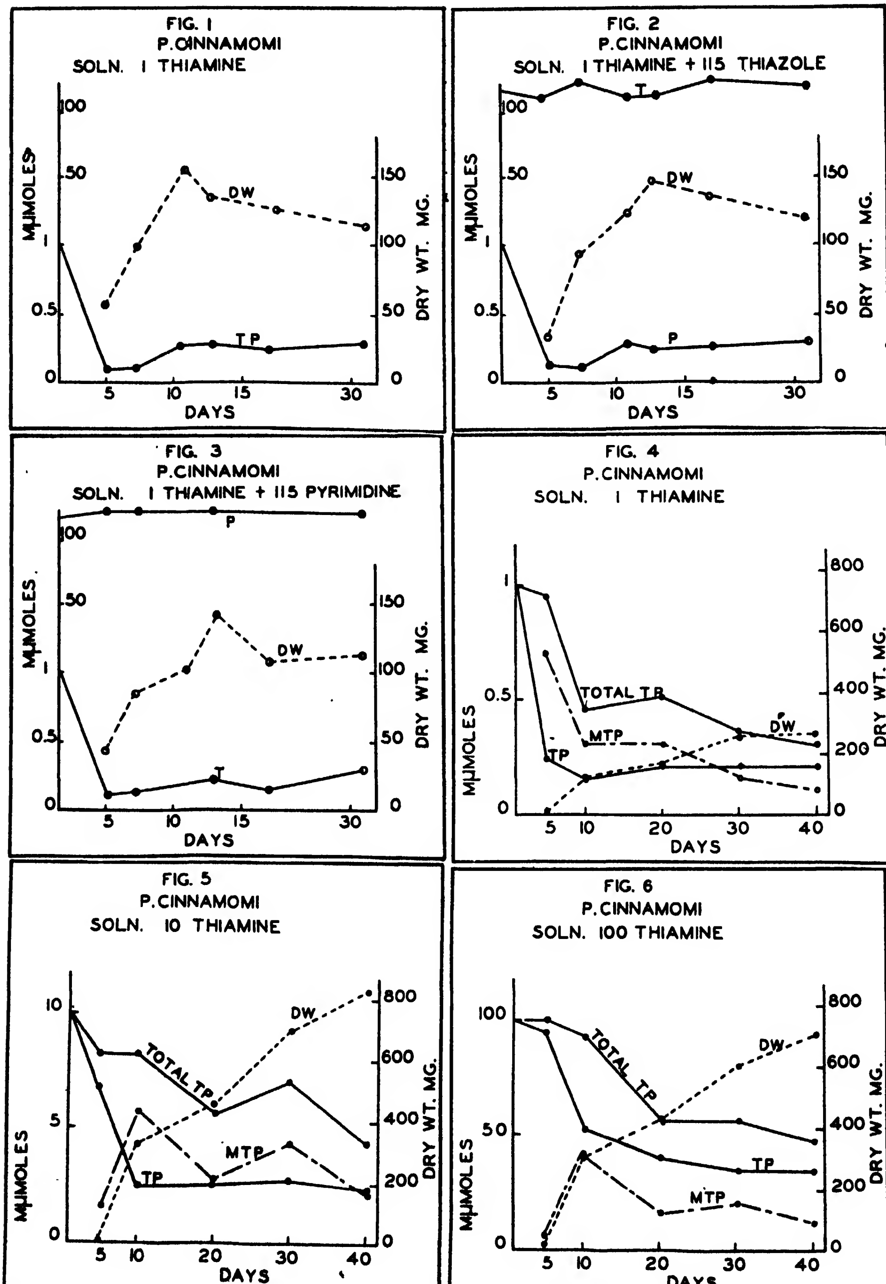
Each assay for thiazole and pyrimidine required the construction of a standard curve from cultures grown for the same time and under the same conditions as the cultures concerned in the assay of the unknown amounts of thiazole and pyrimidine. This was necessary because the temperature of incubation was not entirely constant, the period of growth could not be exactly controlled, and other conditions varied somewhat.

The following example shows the application of the method to solution E 2 in which *P. cinnamomi* had grown for 21 days. One-half the medium from one flask in which *Phytophthora* had grown was added to the analyzing solution containing 115 mμmoles of thiazole. The dry weight of *Phycomyces* mycelium produced in this solution was 58 mg., which lies at 0.15 mμmole on the pyrimidine calibration shown in figure 13. Since the aliquot was one-half, the amount of pyrimidine in the medium was 0.30 mμmole. Thiazole was determined in an aliquot that was 1/50 of the medium from one flask. The weight of *Phycomyces* mycelium was 211 mg., which lies on the thiazole curve at 2.35 mμmoles. This value was multiplied by 50 to find the amount of thiazole in the medium, 118 mμmoles.

The determination of thiazole and pyrimidine was several times more accurate in the E and F series of solutions than in the A series because the larger volume of medium available made possible the use of larger aliquots and more of them. In the E and F series the absolute error in a determination of thiazole or pyrimidine caused by inaccuracy in weights of both the standard and the assay mats of mycelium of *Phycomyces* was estimated to be 0.2 mμmole for amounts less than 1 mμmole and between 10 and 20 per cent for larger amounts.

The mycelia usually were not analyzed since the work of Bonner and Buchman had indicated that little thiamine or free thiazole occurred in the mycelia of *Phycomyces* as old as five days. However, the amounts of thiazole and pyrimidine contained in mycelia were determined in three series of solution E and in all series of solution F as follows: A mycelium was removed from a flask, washed with water, boiled with 25 ml. of 1 per cent hydrochloric acid, allowed to stand for three days, then removed from the hydrochloric acid. The acid solution was neutralized with sodium hydroxide and aliquots were analyzed. Treatment of the mycelium after the first acid extraction with an acid solution of pepsin (10) released insignificant amounts of thiazole or pyrimidine. Apparently the extractable thiazole and pyrimidine were removed by the first treatment.

The validity of the assays depends upon the absence from the solution analyzed of substances other than thiamine and its intermediates which



FIGS. 1–6. *Phytophthora cinnamomi.* FIGS. 1–3. Analysis of solutions E. FIGS. 4–6. Analysis of solutions F and mycelium. DW, dry weight of mycelium; T, thiazole; P, pyrimidine; TP, thiazole or pyrimidine in solution; MTP, thiazole or pyrimidine in mycelium; Total TP, sum of thiazole (or pyrimidine) in solution and mycelium.

would affect the 9–15-day growth of *Phycomyces*. No evidence was found that the four fungi produced substances that increased or decreased the response of *Phycomyces* to thiamine or its intermediates. When an aliquot was doubled, the growth of *Phycomyces* indicated that twice as much thiazole or pyrimidine was present. When a small amount of thiamine was added to an aliquot, the growth of *Phycomyces* was that expected. All of the experimental evidence indicates that the response of organisms to thiamine, thiazole, and pyrimidine is highly specific for the chemical structure of these substances (18).

EXPERIMENTS

1. PHYTOPHTHORA CINNAMOMI

Phytophthora cinnamomi was grown in solutions A and E for 31 days and in solution F for 40 days. The solutions (and the mycelium in series F) were analyzed at intervals for thiazole and pyrimidine. In the A series of solutions 21 flasks, in the E series 18 sets of flasks, and in the F series 15 sets of solutions and mycelia were analyzed.

The results are given in the form of curves (figs. 1–6) which show the dry weight of the fungus, the amount of thiazole and pyrimidine found in the medium at the end of each growth period, and, for solution F, the amount of thiazole and pyrimidine found in the mycelium. The results of all the experiments appeared to be consistent. Curves are presented for the E series because these analyses are considered to be more accurate than for the A series.

Effect of Thiamine and Its Intermediates on Growth of Phytophthora. *Phytophthora* did not grow in any of the basal solutions used in this work unless thiamine was added. The optimum amount of thiamine was greater than 1 and not more than 10 mμmoles per flask. Growth, as measured by increase in dry weight of mycelium, continued for 40 days in the F solutions. It had nearly ceased in the flasks containing 1 mμmole of thiamine but was still fairly rapid in those containing 10 and 100 mμmoles. The growth curves for the A and E series had similar shapes. A maximum dry weight was attained by the 13th day; this was followed by a slow decrease in weight as the mycelium aged. Growth in the solutions containing thiazole or pyrimidine in addition to the thiamine did not differ from that in the thiamine solutions.

Growth nearly doubled when the nutrient solution was diluted with an equal quantity of water. From these results it appears that the efficiency of utilization of thiamine by *P. cinnamomi* is influenced by the concentration of the nutrient solution.

Residual Intermediates in the Culture Solutions. The analyses showed that the thiazole and pyrimidine remaining at any time in the thiamine solutions were present in equal amounts. In the F solutions the amounts of thiazole and pyrimidine remaining after the tenth day were between 20 and

30 per cent of the thiamine added. In the thiamine solutions of the E series, the thiazole and pyrimidine remaining after the fifth day was about 15 per cent of the thiamine originally added. In the thiamine plus thiazole solution about 15 per cent of the pyrimidine and in the thiamine plus pyrimidine about 10 per cent of the thiazole remained after the fifth day of growth. Not enough of the intermediates added to the thiamine solutions disappeared to be detected by the method of analysis. These analyses of the solution in which *Phytophthora* had grown show that the amount of residual thiazole or pyrimidine in the solution was not affected by the presence in the solution of a large amount of the other intermediate. Thiazole and pyrimidine appear to be inert substances for *Phytophthora.* The presence in these solutions of amounts of residual intermediates varying from 10 to 30 per cent (from 0.1 to 30 mμmoles per flask) of the thiamine put in is best explained by assuming that the stock thiamine solutions contained thiazole and pyrimidine in addition to thiamine.[7] It does not seem probable that so large a percentage (and in some series so large an absolute amount) of the thiamine originally present in the solution would not have been absorbed. The thiazole and pyrimidine in the stock solution of thiamine were probably formed by hydrolysis of the thiamine. That different stock thiamine solutions were used in making the E and F series may account for the different residual amounts of thiazole and pyrimidine found in these solutions.

Thiamine Found in the Mycelium. Mycelium grown in thiamine solutions contained thiazole and pyrimidine in equal amounts (figs. 4, 5, 6). The amounts of thiazole and pyrimidine found in the mycelium increased rapidly in the young mycelium, reached a maximum on about the tenth day, then decreased as the mycelium grew. The concentration[8] of the thiazole and pyrimidine in some cultures was found to be as much as 300 times as high in the mycelium as in the solution.

The mycelium had accumulated 40 mμmoles of intermediates from the 100 mμmole thiamine solution by the tenth day (fig. 6). During the next thirty days the intermediates in the mycelium decreased to 10 mμmoles but did not increase in the solution. The use of thiamine by *Phytophthora* did not result in excretion of either thiazole or pyrimidine into the medium. Growth was made at the expense of the accumulated intermediates. A rather large amount of intermediates (30 mμmoles of each) was left in the solution. Thiazole and pyrimidine were accumulated in large amounts by the mycelium only from the thiamine solutions and not from the solutions containing free thiazole or free pyrimidine.

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[7] It is to be remembered that the method of assay determines the total functional amount of an intermediate and does not indicate how much is free and how much is present as thiamine.

[8] The volume in milliliters of the mycelium was estimated to be five times the dry weight in grams since the density of protoplasm is near 1 and the dry weight of a mycelium is approximately 20 per cent of the wet weight.

It is believed that these results can best be explained by assuming that the intermediates found in the mycelium were present primarily in the form of thiamine. This would explain the appearance and disappearance in the mycelium of equimolecular amounts of thiazole and pyrimidine. The presence in the medium of the large amount of residual intermediates can still be explained by the assumption that the intermediates were present as thiazole and pyrimidine which are inert substances for *Phytophthora* until combined to form thiamine.

In the further discussions of the interrelation between thiamine and *P. cinnamomi,* the assumptions will be made that the fungus affects only thiamine and that the intermediates determined in the mycelium exist there mainly as thiamine.

Interpretation of Results. Young, rapidly growing mycelium of *Phytophthora* absorbed the thiamine from the medium, stored it, and used it for further growth. The mycelium did not release to the medium either thiazole or pyrimidine, as was shown by the constant value of the residual intermediates in a solution. That the mycelium did not absorb the free thiazole and free pyrimidine in the solutions was indicated by the presence of residual intermediates in the thiamine solutions and by the analyses of the solutions containing large amounts of free thiazole or free pyrimidine.

2. Phycomyces blakesleeanus

Phycomyces blakesleeanus was grown in solutions of series A for 17 days and in solutions of series E for 31 days. In series A, 36 solutions, in series E, 18 sets of solutions and 6 mycelial mats were analyzed for thiazole and pyrimidine. The dry weights of mycelia and the analyses of solutions of the E series are shown in figures 7, 8, and 9.

Effect of Thiamine and Intermediates on Growth of P. blakesleeanus. *Phycomyces* grew rapidly in the nutrient solutions containing thiamine or both intermediates. It responded to as little as 0.01 mμmole of thiamine or intermediates. The optimum amounts for the solutions used in this work were about 10 mμmoles per flask. The relation between the amount of thiamine or intermediates (in equimolecular amounts) per flask and the dry weight at the end of fifteen days of growth is given by the line marked T in figure 13.

Growth of *Phycomyces* as measured by increase in dry weight occurred in two periods. The first period was from the time of inoculation to the time of the first maximum dry weight which occurred between the fifth and ninth days. After this maximum there was a decrease in dry weight until the second period of growth, which extended from about the twelfth day to the seventeenth day, when a second maximum appeared in the growth curve.

When *Phycomyces* was grown in solutions containing thiamine and a large amount of pyrimidine, the pyrimidine did not affect the growth. *Phycomyces* grown in solutions containing thiazole in addition to thiamine reached a larger maximum dry weight than in the thiamine solutions. The increase in dry weight resulting from the addition of thiazole to a thiamine solution increased with increasing amounts of thiazole until about ten times as much thiazole as thiamine was added, beyond which additional thiazole had no effect. The curve marked P in figure 13 shows the relation between the dry weight produced by *Phycomyces* grown for fifteen days in the presence of a large excess of thiazole and the amount of pyrimidine put into the solution. The maximum dry weight produced in the presence of a large excess of thiazole was between 1.5 and 3 times as much as that produced in the absence of excess thiazole. From these results it is clear that the ratio of thiazole to pyrimidine in the analyzing solutions (never less than 50 to 1) was always large enough to obtain maximum growth.

Intermediates in Culture Solutions. The amount of thiazole in the thiamine solution was very small during most of the experimental period. The amount of pyrimidine in the thiamine solution had reached its lowest value by the fifth day and then increased steadily during the rest of the experiment. Less thiazole than pyrimidine was left in a thiamine solution after *Phycomyces* had grown in it. In the thiamine plus excess pyrimidine solution (fig. 9), the amount of pyrimidine did not change by a detectable amount; the amount of thiazole reached a low value by the fifth day and then did not change very much. In the thiamine plus thiazole solutions (fig. 8), the amount of pyrimidine was relatively constant after the fifth day and was less than the amounts found in the thiamine solutions on the corresponding day. The amount of thiazole decreased rapidly and had reached a constant value by the thirteenth day.

Intermediates in the Mycelium. Very little thiazole was found in the mycelia grown in the thiamine and the thiamine plus pyrimidine solutions. A small amount of thiazole was found in the mycelia grown in the thiamine plus excess thiazole solutions (table 3). Analyses of mycelia grown in thiamine plus thiazole and in thiamine plus pyrimidine solutions showed that the intermediate present in the medium in the larger amount was also present in the mycelium in the larger amount and that the concentrations in solution and mycelium were approximately equal, indicating that the mycelium of *Phycomyces* did not accumulate either thiazole or pyrimidine.

Loss of Thiazole from the Medium. By the fifth day of growth, the dry weights of mycelium produced in the three solutions were nearly the same; and the amount of thiazole missing from the thiamine plus thiazole solution was 75 times as great as that missing from either of the other solu-

FIG. 7
P. BLAKESLEEANUS
SOLN. 1 THIAMINE

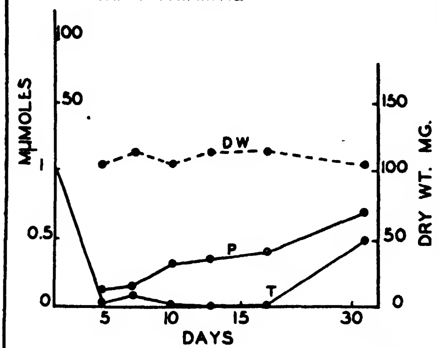


FIG. 8
P. BLAKESLEEANUS
SOLN. 1 THIAMINE + 115 THIAZOLE

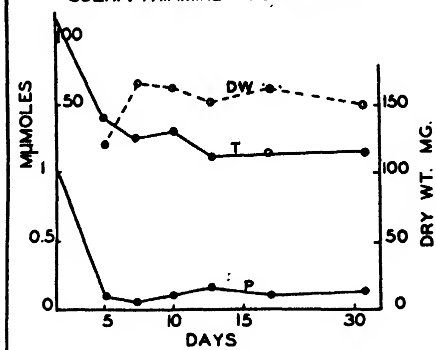


FIG. 9
P. BLAKESLEEANUS
SOLN. 1 THIAMINE + 115 PYRIMIDINE

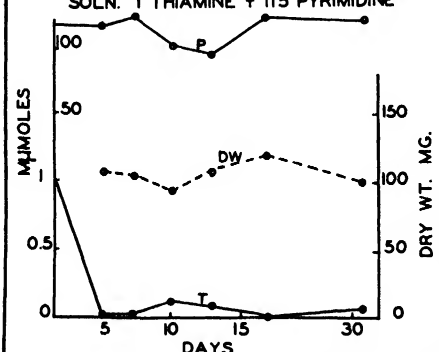


FIG. 10
M. RAMANNIANUS
SOLN. 1 THIAMINE

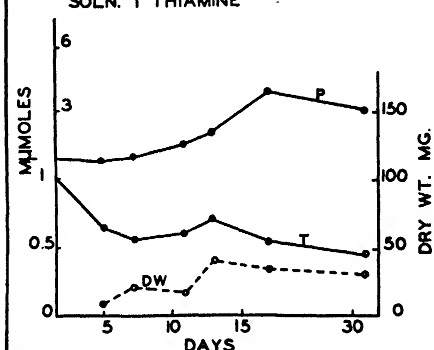


FIG. 11
M. RAMANNIANUS
SOLN. 1 THIAMINE + 115 THIAZOLE

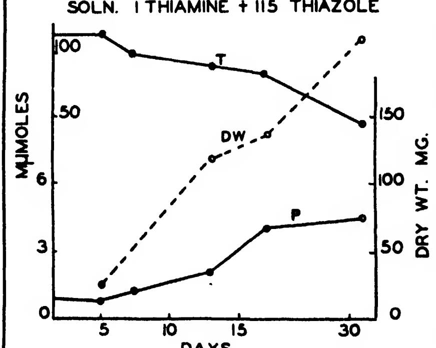
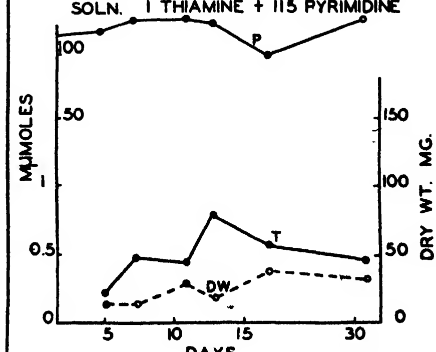


FIG. 12
M. RAMANNIANUS
SOLN. 1 THIAMINE + 115 PYRIMIDINE



FIGS. 7–9. *Phycomyces blakesleeanus*, analysis of solutions E. FIGS. 10–12. *Mucor ramannianus*, analysis of solutions E. DW, dry weight of mycelium; T, thiazole; P, pyrimidine.

tions. In the same time and with the same growth, either 1 mμmole or 75 mμmoles of thiazole could disappear from the medium and not appear in the mycelium. The 75 mμmoles of missing thiazole could not have been removed as a pyrimidine-containing compound because only enough pyrimidine occurred in the medium to combine with 1 mμmole of thiazole. The removal of the large amount of thiazole would seem not to be related to the growth made by the fungus. The thiazole may have been destroyed, it may have been used as a source of organic sulfur, or it may have been combined in a form that was not determined by the method of analysis.

Interpretation of Results. The young and rapidly growing mycelium of *Phycomyces* absorbed the thiamine from the medium and used it during growth. Pyrimidine but not thiazole accumulated in the solution. As a consequence of the presence of the mycelium of *Phycomyces*, thiamine was split into pyrimidine and thiazole, the thiazole disappeared from the solution and mycelium, and pyrimidine accumulated in the solution. If thiazole was present in addition to thiamine, the *Phycomyces* grew somewhat better than it did in the absence of excess thiazole, much of the excess thiazole disappeared from both medium and mycelium, and pyrimidine did not accumulate in the medium. *Phycomyces* grew in the thiamine plus pyrimidine solution as it did in the thiamine solution in the absence of excess pyrimidine, and did not affect the pyrimidine.

3. MUCOR RAMANNIANUS

Mucor ramannianus was grown in solutions of the A and E series for 31 days. In the A series, 21 solutions, in the E series 18 sets of solution were analyzed for thiazole and pyrimidine. The results of the analyses made on solutions of the E series and the dry weight of the mycelia produced in the solutions are given in figures 10, 11, and 12.

Effect of Thiamine and Intermediates on Growth of Mucor. The dry weight of mycelium produced in the solutions containing thiamine and thiamine plus excess pyrimidine were the same; they reached a maximum between the twelfth and eighteenth days and then decreased slowly. In the solutions which received a large amount of thiazole in addition to thiamine, growth increased with time and had not approached a limit when the experiments were terminated at the end of thirty-one days. Growth was about five times as great in this solution as in the ones without extra thiazole. Growth was better in the E solutions containing thiamine or thiamine and pyrimidine than in the corresponding A solutions but was much better in the A solution containing the large amount of thiazole than it was in the corresponding solution of the E series. Since the A solution contained ten times as much potassium dihydrogen phosphate and magnesium sulfate and twice as much mineral supplements as the E solution, growth in the E solution

containing the excess thiazole might have been limited by the amount of minerals.

Intermediates in the Culture Solutions. The amount of thiazole in a thiamine solution in which *Mucor* had grown decreased with increase in the time of growth but did not go much below one-half that originally present (fig. 10). The same was true of the thiazole in the thiamine solution to which pyrimidine was added (fig. 12). The presence of a large excess of pyrimidine in the solution did not affect the final amount of thiazole in the solution if the solution originally contained little thiazole.

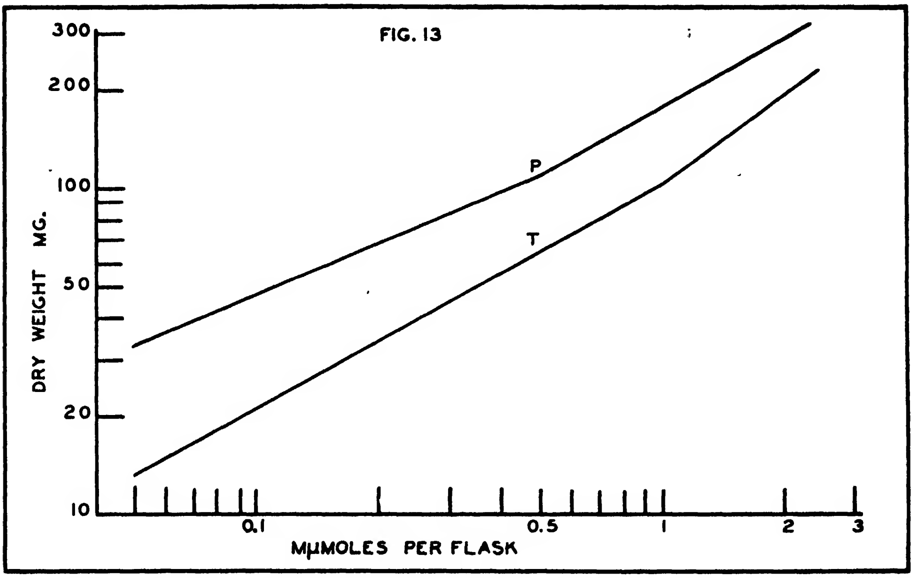


FIG. 13. Relation between the fifteen-day growth of *Phycomyces blakesleeanus* at 25° C. and the amount of intermediate in one flask of the analyzing solution used for determining thiazole, T, and pyrimidine, P.

Since pyrimidine increased in all the solutions in which *Mucor* grew, *M. ramannianus* must have synthesized pyrimidine. In fact *Mucor* synthesized up to nine times as much pyrimidine as was put into the solutions as thiamine. This ability to synthesize pyrimidine explains why *Mucor* grew in nutrient solutions provided with only the thiazole portion of the thiamine molecule. The amount of pyrimidine synthesized may be far in excess of the amount needed to form thiamine with the thiazole present.

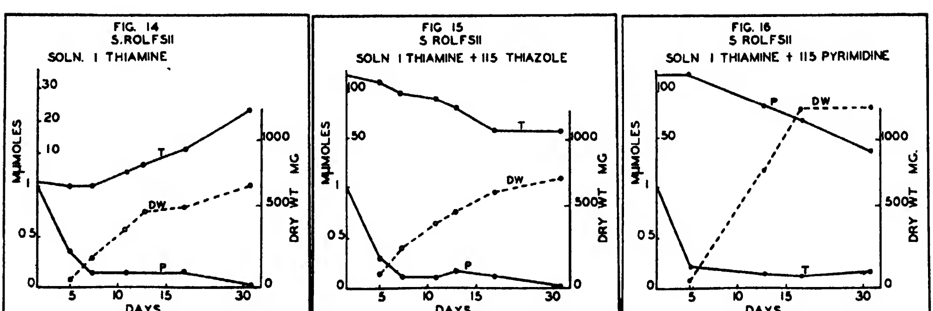
Loss of Thiazole from Nutrient Solutions. A rather large decrease in the amount of thiazole occurred in the solutions which originally contained 115 mμmoles of thiazole (fig. 11). The thiazole not accounted for was several times that needed to combine with the pyrimidine found in the solution. Possibly the missing thiazole was combined with pyrimidine and the thiamine

thus formed was absorbed by the mycelium. If this was true, the analyses gave only the pyrimidine not combined with thiazole and did not indicate the total amount of pyrimidine synthesized. However, the missing thiazole may represent thiazole destroyed or thiazole used as a source of organic sulfur (23).

Interpretations of Results. *Mucor ramannianus* in a thiamine solution absorbs a small amount of thiamine, grows slowly, and synthesizes pyrimidine. The amount of pyrimidine synthesized may be much larger than can be combined with the thiazole present. Excess pyrimidine in the thiamine solution does not affect growth or the synthesis of pyrimidine. *Mucor* growing in a solution containing a large amount of thiazole grows very well, synthesizes pyrimidine, and removes thiazole from the solution in quantities larger than needed to combine with the pyrimidine synthesized. However, the amount of pyrimidine synthesized was approximately proportional to the dry weight.

4. Sclerotium rolfsii

Sclerotium rolfsii was grown in the A and E series of solutions for 31 days. The solutions were analyzed at intervals for thiazole and pyrimidine. Twenty-one flasks of solution A and 18 sets of solutions in the E series were analyzed. The dry weights of the mycelia and the results of the analyses of the E series are shown in figures 14, 15, and 16. The A and E series of solutions gave similar results.



Figs. 14–16. *Sclerotium rolfsii,* analysis of solutions E. DW, dry weight; T, thiazole; P, pyrimidine.

Effect of Thiamine and Intermediates on Growth. *S. rolfsii* grew very well in the thiamine solutions. It produced almost six times as much dry matter in the thiamine solution in 31 days as either *Phycomyces* or *Phytophthora* grown in similar solutions. The growth in the thiamine plus thiazole solution was somewhat better than that in the thiamine solutions. Growth in the thiamine solution to which a large amount of pyrimidine was added was much greater than the growth in thiamine solution. This was expected because *Sclerotium* grew if given the pyrimidine portion of the thi-

amine molecule. The maximum dry weight produced in the solution containing pyrimidine was nearly one-half the weight of sugar put into the solution. Little growth occurred after the 17th day. The growth in the solutions containing thiamine or thiamine plus thiazole seemed to be limited by some factor not sugar, minerals, or nitrogen.

Intermediates in Culture Solutions. The analyses of the thiamine solutions (fig. 14) showed that pyrimidine had decreased by 80 per cent by the 7th day and was not present on the 31st day of growth. The thiazole increased rapidly after the 7th day of growth until by the 31st day 26 times as much thiazole occurred in the solution as was put into it. *Sclerotium rolfsii* synthesized thiazole. The amount of pyrimidine in the thiamine plus thiazole solution (fig. 15) followed much the same course that it did in the thiamine solution. The amount of thiazole decreased during the period of rapid growth (to the 17th day) and then remained unchanged at about 50 per cent (55 mμmoles) of the thiazole put in.

The amount of thiazole in the thiamine plus pyrimidine solutions had decreased by the fifth day of growth to about 20 per cent (0.2 mμmole per flask) of the amount put in as thiamine and remained near that value during the next 26 days. Thiazole was found in the solution at all times. The amount of pyrimidine decreased throughout the growth period. About 30 per cent of the pyrimidine was in the solution when the experiment was terminated. The analyses indicated that growth in the thiamine plus pyrimidine solution was not stopped by lack of thiazole or pyrimidine.

Intermediates in the Mycelium. Thiazole and pyrimidine were determined in mycelia grown in thiamine and in thiamine plus thiazole solutions (table 3). The intermediate present in the solution in the larger amount was present in the mycelium in the larger amount. It was estimated from the relative volumes of mycelium and solution that the concentration of thiazole in the mycelium was the same as in the solution. The thiazole missing from the thiamine plus thiazole solution was not in the mycelium, at least not in a form removed by the methods used in this work.

Loss of Thiazole and Pyrimidine. About half the thiazole put into the thiamine plus thiazole was lost. The loss (55 mμmoles) was rather large and only two per cent of it could possibly be thiazole removed from the solution as thiamine or other compounds containing thiazole and pyrimidine in equimolecular amounts. The thiazole may have been destroyed without functioning in metabolism. Presumably thiazole was synthesized in all solutions even though in two of the solutions, the amount of thiazole decreased with increase in growth.

About 75 per cent (80 mμmoles) of the pyrimidine was lost from the solution which contained the thiamine plus pyrimidine. Although no ap-

parent synthesis of thiazole occurred in this solution, the dry weight of the mycelium indicated that synthesis of thiazole had taken place. Hence the pyrimidine could have been removed as a thiazole-containing compound.

Interpretation of Results. *S. rolfsii* grown in a solution containing a small amount of thiamine removed it fairly rapidly and synthesized thiazole which accumulated in large amounts in the solution. *Sclerotium* grown in a solution containing a large amount of pyrimidine combined it with the thiazole (synthesized by the fungus) to form thiamine which functioned in the metabolism of the fungus. When *Sclerotium* was grown in a solution containing a large amount of thiazole and a small amount of pyrimidine, the pyrimidine eventually disappeared from the solution but only half of the thiazole could be accounted for.

TABLE 3. *The amount of thiazole and pyrimidine in mμmoles recovered from the mycelia of Phycomyces and Sclerotium and in the culture solutions in which these mycelia had grown for 17 and 31 days*

Fungus	Solution	Intermediate	17 days		31 days	
			Mycelium	Medium	Mycelium	Medium
Phycomyces	E 1	thiazole	0.00	0.00	0.06	0.50
		pyrimidine	0.26	0.40	0.08	0.70
	E 2	thiazole	0.76	15	1.04	15
		pyrimidine	0.26	0.08	0.16	0.12
	E 3	thiazole	0.00	0.00	0.09	0.07
		pyrimidine	8.6	135	2.60	120
Sclerotium	E 1	thiazole	2.20	12.5		
		pyrimidine	0.44	0.16		
	E 2	thiazole	4.40	56		
		pyrimidine	0.36	0.12		

DISCUSSION

Thiamine disappears from nutrient solutions in which certain fungi grow and is not completely recoverable from the mycelium; the thiazole portion of the thiamine molecule may be destroyed by some of these organisms. Several explanations for these observations are suggested in the following discussion.

All organisms that have been studied either make thiamine or require an external source of it or its intermediates; hence, it may be inferred that they have a carboxylase system. Thiamine as such or as cocarboxylase may be tightly bound by proteins in the mycelium and not removed by the method of extraction used. The pyrophosphate of thiamine, cocarboxylase, in conjunction with a protein forms carboxylase, an enzyme which decarboxylates pyruvic acid. That cocarboxylase and the protein are very firmly bound was shown by Horowitz and Heegaard (5) for pea root carboxylase and by

Westenbrink, Willebrands and Kamminga (24) for yeast carboxylase. Horowitz and Heegaard found that even denaturation of the protein by boiling it did not release all the cocarboxylase. Other proteins which bind cocarboxylase or thiamine to form nonfunctional (not carboxylase) systems have been demonstrated in certain yeasts (8) and in carp (30). From these results it is to be expected that part of the thiamine not found in the solution and mycelium was actually in the mycelium firmly bound to protein. Possibly the thiamine (about half of that put into the solution) which was neither in the solution in which *Phytophthora* grew nor extracted from the mycelium was bound to protein. Since no evidence for the destruction of pyrimidine by *Phycomyces* and *Sclerotium* was found, the pyrimidine missing from the solution and not recovered from the mycelium (table 3) could have represented bound thiamine. Furthermore, although thiazole was synthesized by *Sclerotium* growing in the thiamine-plus-pyrimidine solution, it did not accumulate in the solution; and at the same time 80 mμmoles of pyrimidine disappeared from the solution; the thiazole and pyrimidine which were not in the medium could have been in the mycelium as thiamine or cocarboxylase bound to protein. *Mucor ramannianus* did not appear to destroy thiazole but what was removed from the solution containing 115 mμmoles of thiazole could have been coupled with pyrimidine synthesized by the fungus and removed as thiamine bound to protein. At least part of the bound cocarboxylase may be used in the normal metabolism of the fungus. It seems probable that fungi contain carboxylase and remove thiamine from the solution in the same way as do pea roots and yeast.

Another way by which thiazole and pyrimidine could have been removed from the solution in equimolecular amounts is by the formation of a compound inactive toward *Phycomyces*, such as thiochrome. Thiochrome occurs in yeasts and might be formed from thiamine by other fungi.

Thiamine which has been hydrolyzed into thiazole and pyrimidine is thiamine destroyed so far as its usefulness to *Phytophthora cinnamomi* or any other organism which cannot synthesize thiamine from the intermediates is concerned. The large residual equimolecular amounts of thiazole and pyrimidine in the thiamine solutions in which *Phytophthora* grew probably represented hydrolyzed thiamine. The thiamine apparently was not split into thiazole and pyrimidine by the fungus but was partially hydrolyzed during the preparation of the culture medium.

A method of inactivating thiazole suggests itself. *Phycomyces*, *Mucor*, and *Sclerotium* possess an enzyme which forms a quaternary ammonium salt of thiazole with pyrimidine, namely thiamine. If the enzyme can function with a compound not pyrimidine, then quaternary ammonium salts of thiazole other than thiamine can be formed. Such compounds, if they were as stable as the methiodide of thiazole (18), would be inactive in the growth of *Phycomyces*; and the thiazole in them would be considered to be destroyed.

Bonner and Buchman (2) have shown that *Phycomyces blakesleeanus* growing in a thiamine solution inactivated the thiazole and released functional pyrimidine. The inactivation occurred only when the thiazole was coupled to pyrimidine to form the quaternary ammonium salt, thiamine. They concluded that the inactivation of thiazole resulted from the opening of the quaternary thiazole ring adjacent to the 2-position. Destruction of the quaternary ammonium salt accompanied the destruction of the thiazole ring and released functional pyrimidine. A compound inactive toward *Phycomyces* was derived from the thiazole. Of the three fungi which synthesized thiamine from thiazole and pyrimidine, *Sclerotium* and *Phycomyces* destroyed thiazole but only *Phycomyces* quickly destroyed small amounts of thiazole. *Sclerotium* synthesized thiazole which did not always accumulate in the solution. The thiazole in the solutions which contained thiamine plus excess thiazole decreased to about one-half of the original amount. The missing thiazole could not have been removed as a pyrimidine-containing compound; there was not nearly enough pyrimidine. It could have been destroyed by opening of the thiazole ring. The results for the thiamine and thiamine-plus-thiazole solutions in which *Sclerotium* grew can be explained by assuming that the fungus synthesizes and destroys thiazole and that the rates are equal when there are about 60 mμmoles of thiazole in the medium. Destruction of thiazole is apparent when more than 60 mμmoles of thiazole are present per culture. It should be remembered that the concentration of thiazole in the mycelium and not the amount in the medium would regulate the rates of synthesis and destruction.

Phytophthora cinnamomi did not synthesize thiamine from the intermediates or destroy thiazole, although Bonner and Buchman (2, footnote 10) seemed to believe that *Phytophthora* destroyed thiamine in the same way as *Phycomyces* does. They say: "Preliminary experiments with *Phytophthora* indicate that this organism (which cannot combine thiazole with pyrimidine) also destroys vitamin with liberation of pyrimidine but does not attack uncombined thiazole." They believed that pyrimidine accumulated in the solutions in which the *Phytophthora* grew. The accumulation of pyrimidine reported by them may have been more apparent than real because their method of using *Phycomyces* to determine pyrimidine neglected the effect of excess thiazole on the growth of *Phycomyces* and gave values for pyrimidine that were from two to three times as high as they would have been by the methods used in this investigation.

If the destruction of thiazole resulted from the use of thiamine in enzyme systems in the protoplasm, other organisms would be expected to destroy it as *Phycomyces* does. Since *Phytophthora* (and possibly *Mucor*) did not destroy thiazole, it would appear that the use of thiamine in its fundamental role as a part of the enzyme system of an organism differs from the process by which thiazole is destroyed by an organism like *Phycomyces*. This indi-

cates that the mechanisms by which thiamine functions in the life processes of *Phytophthora* are distinct from the mechanisms by which thiazole is destroyed by *Phycomyces*. It is of particular interest to note that, of the organisms studied, two of those which synthesize thiamine from thiazole and pyrimidine, namely, *Phycomyces* and *Sclerotium*, destroyed thiazole and the one, *Phytophthora*, which cannot synthesize thiamine from its intermediates did not destroy thiazole. This suggests that the mechanism of destruction of thiazole may be closely related to the synthesis of thiamine from the intermediates.

Two enzyme systems in *Phycomyces* seem to compete for thiamine: carboxylase and the enzyme responsible for the destruction of thiazole. When the growth of *Phycomyces* is limited only by the amount of carboxylase that can be formed, destruction of thiamine results in reduced growth. *Phycomyces* grows better if thiazole is added to thiamine because the thiazole is combined with pyrimidine, which is an end product of the destruction of thiamine, to form more thiamine so that the effective amount of thiamine is greater than the amount originally present (2). The amount of growth obtained in the presence of added thiazole is limited to that obtained when all the pyrimidine has been linked as cocarboxylase to a protein to form carboxylase. The analyses indicate that in solutions containing small amounts of thiamine about one-half of it is destroyed by *Phycomyces*. It seems possible that *Phycomyces* could be placed at unfavorable temperatures at which growth would be reduced or stopped but at which destruction of thiamine might continue.

Mucor ramannianus, in contrast to *Phytophthora* and *Phycomyces*, can meet its thiamine requirements if it is given thiazole because it makes pyrimidine and couples the two intermediates to form thiamine. Whether or not *Mucor* destroys thiazole as *Phycomyces* does was not demonstrated by the methods used. *Sclerotium rolfsii* differs from the other three fungi in its ability to synthesize thiazole but is like *Phycomyces* in its ability to destroy it.

The four fungi differed greatly in their response to 1 mμmole of thiamine; they gave maximum dry weights ranging from 40 to 1240 mg. Does this mean that different organisms have greatly different ratios of carboxylase to living protoplasm? Or does it mean that in one organism the cocarboxylase is firmly bound to its protein while in another as the old parts of the mycelium die the cocarboxylase is released and can be used to form new protoplasm? Another possibility is that organic substances, in addition to thiamine, limit the growth, though the influence of different amounts of mineral elements must not be overlooked (16). The effect of the concentration of minerals on mobility of cocarboxylase and destruction of thiamine is worth investigating.

SUMMARY AND CONCLUSIONS

1. *Phycomyces blakesleeanus* destroyed thiamine with the liberation of pyrimidine and destroyed thiazole. An excess of thiazole added to a thiamine solution increased growth but an excess of pyrimidine did not influence growth.

2. *Phytophthora cinnamomi,* apparently, utilized thiamine without destroying thiazole or pyrimidine. No more pyrimidine than thiazole accumulated in the medium. Growth in a thiamine solution was not influenced by the amount of thiazole or pyrimidine in the solution.

3. *Mucor ramannianus,* when grown in a solution containing thiamine or one containing thiamine and thiazole stopped growing before more than half the thiazole was removed. *M. ramannianus* synthesized pyrimidine. Good growth was obtained only in solutions containing a large amount of thiazole.

4. *Sclerotium rolfsii,* grown in solutions containing thiamine, synthesized considerable amounts of thiazole. *Sclerotium* apparently destroyed thiazole. This fungus grew much more in the thiamine solution than any of the other fungi.

5. The four organisms used in this work have thiamine requirements that are not influenced by the composition of the medium. All of them except *Mucor* grew better in the dilute than in the concentrated mineral solution. When the solution contained a large amount of thiazole, *Mucor* grew best in the strong mineral solution.

6. The destruction of thiazole may not be related to the use of thiamine in metabolism but rather to the ability of the fungus to synthesize thiamine from the two intermediates.

I wish to express my thanks to Dr. W. J. Robbins for his helpful suggestions and criticisms during this investigation.

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(See also under Genetics: **Johnson**)

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INDEX TO VOLUME 69

New names and combinations are designated by **bold face** type.